FUNCTIONAL DIVERSITY AND INTERACTIVE EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON THE PERFORMANCE OF LEGUMES

SUMMARY
OF THE
THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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IN

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BY

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SUMMARY

In high input agricultural practices huge quantities of agro-chemicals including both fertilizers and pesticides are applied regularly but injudiciously in order to accomplish maximum crop production. Of these, relatively higher amounts of unutilized fertilizers persists in soil and may cause toxicity to soil microflora/fauna, waters and consequently foods and via different food chains to human health. Therefore, the sustainability in agricultural systems without compromising the environmental quality and conservation has become one of the major concerns around the world. So, due to the spiraling costs and severe toxicity to foods, water and environment resulting from the indiscriminate application of fertilizers it has become even more imperative to discover some inexpensive alternative to meet out such challenges. In this context, plant growth promoting rhizobacteria indeed has provided some solutions to the problems. Considering the beneficial impact of microbial communities and inadequate and conflicting reports available on the use of microflora in different production systems, this investigation was aimed at identifying some novel plant growth promoting rhizobacteria with multiple qualities. Subsequently, the molecularly characterized and best of the lot in terms of plant growth promoting activities were used to inoculate legumes such as chickpea, pea, greengram and lentil, and the impact was observed both in pot and field soils treated with or without synthetic fertilizers. To achieve these, the present investigation was therefore, designed with specific following objectives:-

(i) to assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area

(ii) isolation of N_2 fixing bacteria from the nodules of legumes grown in conventional soils and P- solubilizing bacteria from different rhizospheric soils

(iii) to isolate ACC deaminase producing bacterial strains from different rhizospheres

(iv) to assay the production of plant growth promoting substances by the PGPR strains both qualitatively and quantitatively

(v) to characterize the PGPR strains morphologically, biochemically and by 16S rRNA gene sequence analysis

(vi) assessment of the impact of microbial inoculants on the performance of chickpea, pea, greengram and lentil grown in sandy clay loam soils treated with/without nitrogenous/phosphatic fertilizers and

(vii) nutrient uptake analysis in the test legumes

The rhizospheric soils of mentha, chilli, cabbage, mustard, chickpea, pea, greengram, and lentil, grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were used to determine microbial diversity. The viable counts of bacteria, fungi and actinomycetes differed considerably among rhizosphere soils. Generally, the total bacterial populations was highest ($4.28x10^7$ cfu/g soil) while those of actinomycetes was lowest ($1.6x10^4$ cfu/g soil) in all soil samples tested. The order of microbial population in all soil samples was found as: bacteria>fungi>actinomycetes. Among different rhizospheres, the bacterial populations was recorded lowest ($3.42x10^7$ cfu/g soil) in cabbage rhizosphere while in chickpea, pea, greengram and lentil it was $3.62x10^7$, $2.71x10^7$, $4.21x10^7$ and $3.94x10^7$ cfu/g soil, respectively. The rhizospheric soils of mentha, however, showed a considerable increase of 21, 25, and 11% in bacterial populations compared to those recorded for chilli, cabbage, and mustard, respectively. The fungal populations in all the rhizospheric soils ranged from $1.1x10^5$ (lentil) to $1.8x10^5$ (mentha) cfu/g soil. The populations of asymbiotic N_2 fixer (ANF) varied noticeably among rhizosphere soils. The ANF in rhizospheric soils ranged between $1.9x10^5$ cfu/g soil (mustard) to $3.2x10^5$cfu/g soil (pea). Moreover, the populations of PSB were greater (mean value $5.24x10^5$ cfu/g soil) in all samples than theP S fungi ($5.20x10^3$ cfu/g) . Similarly, the PSF counts were recorded highest in pea ($6.8x10^3$ cfu/g soil) and lowest in mentha ($3.2x10^3$ cfu/g) rhizospheric soils. While comparing the PSM (including bacteria and fungi) populations in all the rhizosphere soils, the order was: greengram>pea>mentha>chickpea>lentil>chilli>cabbage> mustard. Furthermore, the isolated bacterial cultures showed a variable morphological and biochemical characteristics. Generally, the rhizobial strains were Gram negative while PSB showed a variable Gram reaction. Rhizobial strains in general were positive to all the biochemical reactions except methyl red, Voges Proskauer, indole and gelatin hydrolysis test. In contrast, the PSB showed a considerable variation in biochemical properties. Among the bacterial strains, 38% each of *Mesorhizobium* spp. (chickpea) and *Rhizobium* spp. (pea), 33% each of *Bradyrhizobium* spp. (greengram) and *Rhizobium* spp. (lentil), 40% *Azotobacter* spp. and 36% of PSB were tested further for

evaluating the synthesis of ACC deaminase, phosphate solubilization, IAA, production of siderophores, ammonia, hydrogen cyanide and EPS and antifungal activity. Based on the PGP activities observed under *in vitro* conditions, the mesorhizobial strains were grouped into four PGP groups. All strains of *Mesorhizobium* produced IAA, NH_3 and EPS while 67% strains showed ACC deaminase activity. A total of 47% mesorhizobial strains had both siderophore and HCN activity. Of these, 13% demonstrated both P-solubilization and antifungal activity. The PGP group I included one strain (RG5) which showed 8 PGP traits followed by group II, which had only one strain (RG4) positive to ACC deaminase, IAA, siderophore, NH_3, HCN, EPS and antifungal activity. In PGP group III, 5 strains exhibited a positive reaction to ACC deaminase, IAA, siderophore, NH_3 and EPS, while PGP group IV had 3 bacterial strains showing positive reaction to ACC deaminase, IAA, NH_3, and EPS. The PGP group V, had only one strain (RG6) which showed P- solubilization, IAA, synthesize NH_3 and EPS while PGP group VI included four strains positive for IAA, NH_3, and EPS. All *Rhizobium* strains isolated from pea nodules produced IAA, NH_3 (100%) and EPS where as only 47% strain could synthesize ACC deaminase and HCN. Siderophores, antifungal activity and P-solubilizing activity was shown by 33, 40, and 13% strains, respectively. Similarly, *Rhizobium* strains isolated from pea nodules were grouped into three PGP groups. The PGP group I included two strains (RP2 and RP6) with 7 PGP followed by PGP group II, which had 3 strains positive to ACC deaminase, IAA, siderophore, NH_3, HCN, and EPS. Two strains in PGP group III, displayed a positive reaction to ACC deaminase, IAA, synthesis of NH_3, EPS and antifungal activity while group IV included only one strains capable of secreting IAA, NH_3, HCN and EPS. The PGP group V included 6 strains which synthesized IAA, NH_3 and EPS. Interestingly, all strains of *Bradyrhizobium* were able to synthesize NH_3 and EPS while IAA was produced by 90% strains. Siderophores, HCN, ACC deaminase activity, P-solubilization, and antifungal activity were shown by 50, 50, 20, 10 and 30%, respectively. Similarly, other PGPR were divided into different functional groups.

Rhizobia including *Mesorhizobium* (chickpea nodules), strains of *Rhizobium* (pea nodules), *Bradyrhizobium* (greengram nodules), and *Rhizobium* (lentil nodules) and P-solubilizing bacteria were positive for ACC deaminase activity. The ACC deaminase activity among *Mesorhizobium* ranged from 113 µmol α-ketobutyrate/mg protein/h (RG8) to 258 µmol α ketobutyrate/mg protein/h (RG4) while among *Rhizobium* it

differed between 132 µmol α ketobutyrate/mg protein/h (RP10) to 238 µmol α-ketobutyrate/mg protein/h (RP2). *Bradyrhizobium*, and *Rhizobium* isolated from greengram and lentil nodules, respectively were positive to ACC deaminase. strain PSE9 of PSB produced 227 µmol α ketobutyrate/mg protein/h while strain PSE3 could synthesize 625 µmol α ketobutyrate/mg protein/h. *Achromobacter* sp. ES1 produced 163 µmol α ketobutyrate/mg protein/h) and *Pseudoxanthomonas* sp. strain ES5 (578 µmol α ketobutyrate/mg protein/h), respectively. Moreover, a total of 14% rhizobacterial strains showed PS activity and produced halo on solid Pikovskaya which ranged from 6 mm (*Rhizobium* sp. RP6) to 10 mm (*Mesorhizobium* sp. RG5). The SI value for rhizobia ranged from 1.4 (*Bradyrhizobium* sp. RB9) to 2.1 (*Bradyrhizobium* sp. RB6) while for *Azotobacter* spp. it was 1.4 (AZ 20) to 2.6 (AZ 19) and for P-solubilizing bacteria the S.I. varied between 1.5 (*Bacillus* sp. PSE16) to 3.8 (*P. putida* PSE5). The solubilizing efficiency (S.E.) of each P-solubilizer differed from 42 (*Bradyrhizobium* sp. RB9) to 116 (*Bradyrhizobium* sp. RB6) for rhizobia, 50 (*Azotobacter* AZ20) to 150 (*Azotobacter* AZ5) for *Azotobacter* spp. and 50 (*Enterobacter* PSE15) to 333 (*Pseudomonas* PSE3), respectively. The amount of P-solubilized by rhizobia ranged from 45 µg/ml (*Rhizobium* sp. RP6) to 148 µg/ml (*Bradyrhizobium* sp. RB6), 87 µg/ml (*Azotobacter* sp. AZ20) to 215 µg/ml (*Azotobacter* sp. AZ19) among non-symbiotic N_2 fixers and 111 µg/ml (*Enterobacter* sp. PSE26) to 321 µg/ml (*Achromobacter* PSE28). In addition, the solubilization of TCP by different bacterial cultures was coupled with consequent decrease in pH values that ranged between 5.7 (*Rhizobium* sp. RV9) to 6.1 (*Mesorhizobium* sp. RG6) , 5.2 (*Azotobacter* AZ10 and *Azotobacter* AZ19) to 5.9 (*Azotobacter* AZ1) and 4.4 (*Bacillus* PSE21) to 5.8 (*Enterobacter* PSE30).

The production of IAA by the selected bacterial genera assayed in LB broth treated with (100 µg/ml) or without (0 µg/ml) tryptophan varied among treatments. The amount of IAA synthesized by mesorhizobial strains varied between 14 (RG14) to 29 µg /ml (RG10) in LB broth without tryptophan and 32 (RG14) to 75 µg /ml (RG4) in LB broth supplemented with 100 µg/ml tryptophan. Among the pea specific *Rhizobium* isolates, strain RP9 produced a maximum amount of 32 (0) and 73 µg/ml IAA (100 µg tryptophan/ml). The amount of IAA synthesized by rhizobial strains varied between 13 (RP15) to 32 µg /ml (RP9) at 0 µg/ml tryptophan and 41 (RP3) to 75 µg /ml (RP8) at 100 µg/ml tryptophan, respectively. *Bradyrhizobium* strains also

produced a significant amount of IAA, maximum being 95 µg/ml IAA by the strain RB4 followed by 85 µg/ml IAA at 100 µg/ml tryptophan, . Similarly, the *Rhizobium* strains isolated from lentil nodules showed a variable amount of IAA. Of the *Azotobacter* sp., strains AZ19 and AZ4 were most effective and produced 96 and 89 µg/ml IAA at 100 µg/ml tryptophan, respectively. Among P-solubilizers, PSE25 maximally produced IAA (62 µg/ml) which was followed by PSE24 (62 µg/ml IAA). Generally, the synthesis of IAA by all molecularly characterized P-solubilizers was greater when grown in medium treated with tryptophan than those recorded for untreated medium. In yet other study, the production of IAA was increased with increasing concentration of tryptophan but there were little difference in the synthesis of IAA between the incubation intervals among rhizobial strains. On CAS agar plates, a total of 47% of the *Mesorhizobium* strains produced a visible orange yellow halo on CAS agar plates after five days of incubation whose size varied between 10 (*Mesorhizobium* RG1) to 12 mm (*Mesorhizobium* RG3 and RG8). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain RG8 yielded 16 and 33 µg/ml of 2,3-dihydroxy benzoic acid (DHBA) and salicylate (SA), strain RG7 produced 15 and 34 µg/ml of DHBA and SA, strain RG5 yielded 13 and 28 µg/ml of DHBA and SA, and strain RG4 produced 15 and 29 µg/ml of DHBA and SA, respectively. Similarly, 33% of the pea rhizobia showed an orange yellow colored zone which was greater than 10 mm in size. Additionally, among the siderophore positive rhizobial strains, strain RP6 maximally enhanced the DHBA by 25% relative to the poorly DHBA secreting strain RP2 while strain RP10 showed maximum increase in SA (63%) compared to ~~the~~ lowest SA producing strain RP3. Similarly, strains of *Bradyrhizobium* species showed orange yellow colored zone of varying sizes after five days of incubation. Strain RB3 considerably enhanced DHBA by 14% while it increased the SA by 40% in comparison to the lowest siderophore synthesizing strain RB6. In a similar manner, *Rhizobium* species isolated from lentil nodules, *Azotobacter*, *Achromobacter*, *P. putida*, *Enterobacter*, *B. pumilus*, *Pseudoxanthomonas*, and *Stenotrophomonas* showed variable amounts of siderophores. Additionally, the strains of mesorhizobium, rhizobia, *Bradyrhizobium*, *Azotobacter* and PSB were positive to EPS, NH_3 and cyanogenic compounds.

Antifungal activity of N_2-fixers (N=70) and P-solubilizers (N=30) assessed on PDA differed considerably against three phytopathogens, namely, *Rhizoctonia* sp.,

Penicillium sp. and *Alternaria* sp. Also, *Azotobacter* sp., and few strains of P-solubilizers inhibited the growth of test phytopathogens. The sensitivity/resistance profile of N_2-fixers and P-solubilizers determined using disc diffusion method was variable. On the basis of molecular characteristics, some of the bacterial strains were identified as *Pseudomonas putida* strain PSE3 and PSE5 (Gene Bank accession number HM236047 and HM236047), *Achromobacter* strain ES1 and ES6 (Gene Bank accession number JX483710 and JX 965905), *Enterobacter* strain ES2 (Gene Bank accession number JX 965901) *Bacillus pumilus* strain ES3 (Gene Bank accession number JX 965902), *Pseudoxanthomonas* strain ES4 (Gene Bank accession number JX 965903) and *Stenotrophomonas* strain ES5 (Gene Bank accession number JX 965904). Later on, phylogenetic tree of eight P-solubilizer strains was constructed.

Considering the importance of soil microbes in enhancing the crop production, and expression of multiple growth promoting activities by PGPR strains as observed here, some of the potential PGPR strains were further used to assess their impact on chickpea, pea, greengram and lentil grown under both pot and field soils treated with/without recommended rates of urea and diammonium phosphate (DAP). The recommended rates of urea and DAP in general, did not have any significant ($P \leq 0.05$) effect on the biological and chemical properties of chickpea, pea, greengram and lentil grown in alluvial soils compared to those of single or composite application of microbial cultures. Of the two fertilizers, DAP showed a profound impact on the measured parameters of the legumes. For example, *P. putida* among sole inoculation, had an obvious stimulatory effects on dry matter accumulation in all legumes and enhanced the total dry biomass of chickpea by 8 (pot) and 7% (field), pea dry matter yield by 12 (pot) and 8% (field), greengram biomass by 15 (pot) and 17% (field) and lentil biomass by 8 (pot) and 13% (field) at harvest over DAP. Microbial cultures in the presence of recommended rates of urea and DAP further increased the whole biomass of each legume. The co-culture of *P. putida, Bacillus, Azotobacter* and [*M. ciceri* (chickpea)], [*R. leguminosarum* (pea)], *Bradyrhizobium* sp. (vigna) and *Rhizobium* sp. (lentil) strains showed a more profound impact on biological and chemical characteristics of chickpea, pea, greengram and lentils. The P-solubilizers (*P. putida, B. pumilus* and *Azotobacter*) when used in association with N-fixers (rhizobia, *Mesorhizobium/Bradyrhizobium*) had the most identifiable effects and tremendously increased the chlorophyll contents of each legume relative to other inoculated/uninoculated plants grown in soils treated with/without fertilizers. As an

example, pea plants co-inoculated with [*B. pumilus* with *R. leguminosarum*] had the highest chlorophyll content in foliage of both pot and field grown plants compared to uninoculated and untreated control. Similarly, the co-cultures of *Bradyrhizobium* with [*P. putida*]. [*B. pumilus*] and [*Azotobacter*] maximally increased the chlorophyll content in fresh foliage of greengram by 35, 37 and 33%, respectively as compared to control plants grown in pot while the chlorophyll content in field crops was increased by 37, 39 and 34%, respectively. The symbiotic characteristics (nodulation and leghaemoglobin content) of inoculated/uninoculated chickpea, pea, greengram and lentil plants grown in soils treated with or without urea/DAP was variable. Among fertilizer, DAP in general showed increasing effect on nodulation and leghaemoglobin over urea but it was statistically non significant ($P \leq 0.05$). The sole application of rhizobia specific to each legume in contrast remarkably increased the symbiotic characteristics relative to other single microbial cultures or sole application of urea/DAP, both in pot and fields. As an example, rhizobia when used alone, significantly enhanced the nodulation in (i) chickpea grown in pot (58%) and field (69%) (ii) pea by 14 (pot) and 35% (field) (iii) greengram 49 (pot) and 57% (field) and (iv) lentil by 27 (pot) and 38% (field) over single application of DAP. The leghaemoglobin content in each rhizobia inoculated legumes were higher than those recorded for fertilizer treated/other culture treatments. The co-culture of *B. pumilus* with rhizobia in particular performed exceptionally well and enhanced the nodulation and leghaemoglobin content profoundly compared to other microbial or fertilizer treatments . For example, the composite inoculation of [*Rhizobium* with *B. pumilus*] significantly increased the NN, NDB and Lb content in fresh nodules by 86, 91 and 86% (pot experiment) and by 169, 95 and 94% in field grown peas above the control at 90 DAS, respectively. Inoculation of legumes with PSB and N_2 fixers (both rhizobia and *Azotobacter*) considerably increased the N and P accumulation within roots and shoots of chickpea, pea, greengram and lentil plants grown in soils treated with/without chemical fertilizers. Moreover, the combined inoculation effects were greater than the sum of the individual inoculation effects, suggesting synergism beyond simple additive effects (positive multiplicative interaction). For example, the highest increase in concentrations of N and P was recorded with *B. pumilus* with *Bradyrhizobium* in root and shoots of greengram plants over DAP application. In contrast, the application of *Azotobacter* with rhizobia in general had a poor impact on N and P contents of both roots and shoots of all legumes. *Pseudomonas putida*, *B.*

pumilus, *Azotobacter* and *Rhizobium* when used alone, significantly ($P \leq 0.05$) enhanced the N contents in roots and shoots of field grown lentil plants by 38 and 56%, 43 and 61%, 29 and 54% and, 52 and 72%, respectively relative to control . The P concentration in roots and shoots of lentil plants grown in pots following sole application of *P. putida* and *B. pumilus*, *Azotobacter* and *Rhizobium* was massively increased by 52 and 48%, 64 and 58%, 36 and 42% and, 64 and 55%, respectively, over control. The co-culture of [*Rhizobium* and *B. pumilus*] markedly augmented the N concentration in roots and shoots by 105 and 76% (pots) and 86, 95% (field) while P content in roots and shoots of pot grown lentil was enhanced by 104 and 77% and in field grown plants it was 104 and 76% above pot/field control plants. The impact of mixture of both urea (30 kg/ha) and DAP (90 kg/ha) on the measured parameters was statistically significant compared to other single treatment of urea or DAP or control plants . Of the two fertilizers, 90 kg DAP/ha in general, produced maximum positive effect on the measured parameters of either inoculated or un-inoculated lentil plants.

The impact of fertilizers and microbial inoculations on both quantity and quality of chickpea, pea, greengram and lentil grains was assessed in pot/field experiments. There were no significant difference among the two fertilizers in terms of seed yield or grain protein of chickpea, pea, greengram and lentil at harvest. In contrast, the sole application of microbial cultures like *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* specific to each legume exhibited superior impact over single application of either urea or DAP on the measured parameters. As an example, *B. pumilus* enhanced the seed yield by 13 (pot) and 24% (field), pea yield by 8.3 (pot) and 14% (field), greengram yield by 13 (pot) and 26% (field) and lentil seed by 36 (pot) and 39% (field) at harvest. The seed yield was further enhanced due to dual inoculation of *B. pumilus* and *Rhizobium* specific to each legume compared to fertilizers (both independent and mixture) and single and other multiple inoculation treatments. For example, *B. pumilus* with *M. ciceri* (chickpea) *B. pumilus* with *R. leguminosarum* (pea) *B. pumilus* with *Bradyrhizobium* (greengram) and *B. pumilus* with *Rhizobium* sp. (lentil) increased the yield by 22 (pot) and 16% (field), 13 (pot) and 14% (field), 12 (pot) and 6% (field) and 8 (pot) and 6% (field) over urea with DAP. While comparing the impact of all treatments on seed yield, the grain yield of pea plants for instance recorded for field trials following inoculation or fertilizer application increased in the order: *P. putida* +

Rhizobium>urea+DAP=urea+*P.putida*>DAP+*Rhizobium*>*Rhizobium*>*Pseudomonas* >urea=*DAP*. No significant impact of any treatment on grain protein of any legume was observed except some legumes where marginal increase was noticed following microbial inoculation. All the measured parameters were strongly and positively correlated.

In conclusion, the mixed inoculations of plant growth promoting rhizobacteria with exceptional qualities as observed here, especially the N_2 fixers and P solubilizers, improved the plant vitality, grain quality and showed a dramatic increase in grain yield of chickpea, pea, greengram and lentil, both under pot and filed conditions. Since legumes require a considerable amount of important but scarce plant nutrients, inoculation with favorably interacting PGPR strains are likely to provide an inexpensive alternative to chemical fertilizers for raising the overall performance of chickpea, pea, greengram and lentil, in different production systems. Moreover, this microbial approach if implemented properly will help to reduce toxicities of chemical fertilizers to soil fertility, waters and foods across different ecological niches.

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"Functional diversity and interactive effect of plant growth promoting rhizobacteria on the performance of legumes"** has been carried out by **Mr. Ees Ahmad**, under my supervision. The work included in this thesis is original and has not been submitted for any other degree. The work is suitable for the award of Ph.D. degree in (Ag.) Microbiology of Aligarh Muslim University, Aligarh.

Date:

Dr. Mohd. Saghir Khan
(Supervisor)

December, 31, 2013

DECLARATION

This thesis entitled "**Functional diversity and interactive effect of plant growth promoting rhizobacteria on the performance of legumes**" contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made.

Ees Ahmad

(Ees Ahmad)

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Ees Ahmad

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List of Abbreviations

-	Negative
%	Percent
°C	Degree centigrade
+	Positive
±	Standard deviation
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
BSA	Bovine serum albumin
CAS	Chrome Azurol S
CFU	Colony forming unit
Chl	Chlorophyll
cm	Centimeter
DAE	Days after emergence
DAP	Diammonium phosphate
DAS	Days after sowing
df	Degree of freedom
DHBA	2, 3-Dihydroxy benzoic acid
ePGPR	Extracellular plant growth promoting rhizobacteria
EPS	Exopolysaccharides
g	Gram
h	Hour
HCN	Hydrogen cyanide
IAA	Indole acetic acid
iPGPR	Intracellular plant growth promoting rhizobacteria
kg	Kilogram
L	Liter
Lb	Leghaemoglobin
LSD	Least significant difference
M	Molar
mg	Milligram

ml	Milliliter
mM	Milimolar
MRL	Maximum resistance level
N	Nitrogen
nm	Nanometer
P	Phosphate
PGPR	Plant growth promoting rhizobacteria
PSB	Phosphate solubilizing bacteria
PSM	Phosphate solubilizing microorganism
rDNA	Ribosomal deoxyribonucleic acid
RP	Rock phosphate
rpm	Revolutions per minute
s	Second
SA	Salicylic acid
SI	Solubilization index
TCP	Tri-calcium phosphate
v/v	Volume per volume
X	Recommended dose
YEM	Yeast extract mannitol
µg	Microgram
µl	Microlitre
µM	Micromolar

In contemporary agricultural practices millions of tons of agro-chemicals (fertilizers and pesticides) are frequently but indiscriminately used to achieve optimum crop yields. Such synthetic chemicals are however, not completely used up by plants and hence, persists in different forms in soil. From here, they leach deep into the grounds and disrupt the composition and functions of beneficial rhizosphere microorganism (Ai et al., 2012), soil matrix (Ai et al., 2013; Lemanski and Scheu 2014) and via food chain, the human health (Ayala and Rao 2002). Comparing the fertilizer use efficiency of different countries, India rank the second largest consumer of chemical fertilizers in the world, after China which is expected to increase further to the tune of about 41.6 billion tonnes by the year 2020 (Sharma and Thaker 2012). Furthermore, the chemical fertilizers including both nitrogenous (e.g., urea) and phosphatic (e.g., single super phosphate) fertilizers are quite often used either alone (Maheshwari et al., 2010) or as mixture (Malhi et al., 2007) for example, the use of diammonium phosphate (DAP) for enhancing the crop production in different soil ecosystems. The excessive use of agro-chemicals is however, posing some serious threat to the environments. Therefore, the sustainability in agricultural systems without compromising the environmental quality and conservation has become one of the major concerns of the scientists working in different agronomic area around the world. So, due to the alarmingly very high costs of fertilizers and some acute environmental hazards associated with their use (López-Bellido et al., 2013), it has become increasingly important to find some low cost alternative like the use of renewable resources which could both be inexpensive and could minimize the environmental threats (Bashan 1998; Vessey 2003; Adesemoye et al., 2009). In this context, the discovery of plant growth promoting rhizobacteria (Kloepper et al., 1986; Arshad and Frankenberger 1998; Zahir et al., 2004; Khalid et al., 2009; and Ahemad Khan 2011a; Oves et al., 2013) has provided some relief to the poor agronomic practitioners largely due to low cost and easy and abundant availability. And, during the last couple of decades, there has been some practical progress in this direction where some new and functionally exciting/novel microbes have been used for enhancing agriculture productivity in a more sustainable manner. Also, advances in understanding some finer details of functionally diverse soil microflora together with advent of some molecular tools have made things easier where naturally abundant yet functionally divergent group of microbes could be explored for improving crop productivity, and in turn, has offered

an economically attractive, ecologically sound and economically inexpensive alternative to chemical fertilizers.

Among the beneficial soil microflora, especially the PGPR that may involve both nodule bacteria (Naz et al., 2009a) and some other free living growth promoting rhizobacteria, when applied to seeds and soil have been found to facilitate the plant development by- (i) supplying hugely important nutrients to plants (Sashidhar and Podile 2010) (ii) releasing phytohormones for example, IAA (Naz et al., 2009b; Kavamura et al., 2013), gibberellins (Cassan et al., 2009) and cytokinin (Cassan et al., 2013) (iii) solubilizing/mineralizing complex inorganic/organic P to available P (Richardson et al., 2009; Khan et al., 2013) (iv) alleviating the stress induced by ethylene on plants by synthesizing 1-aminocyclopropane -1-carboxylate (ACC) deaminase to reduce ethylene level (Ahmad et al., 2012) (v) producing siderophores for iron sequestration (Roca et al., 2013) and cyanogenic compounds (Ghyselinck et al., 2013) and (vi) by releasing various other metabolites/ antimicrobial compounds which could inhibit the growth of phytopathogens (Khan et al., 2002; Khan et al. 2009; Sambanthamoorthy et al., 2012). These bacteria after inoculation rapidly colonize onto the seeds and root surfaces in response to exudation (Frankenberger and Arshad 1995; Glick et al., 1998; Khalid et al., 2006) and hence, exhibit positive, neutral or negative effect onto the plant growth. A diverse array of bacteria including species of *Rhizobium* (Hungria et al., 2013) *Bradyrhizobium* (Prévost et al., 2012), *Pseudomonas* (Jha et al., 2011), *Azospirillum* (Cassan et al., 2009), *Azotobacter* (Gaytan et al., 2012), *Bacillus* (Basharat et al., 2009), *Enterobacter* (Mehnaz et al., 2001; Collvino et al., 2010), *Aeromonas* (Mehnaz et al., 2001), *Burkholederia* (Mamta et al., 2010) *Serratia* (Dastarager et al., 2011), *Pantoea* (Mishra et al., 2011) and many others, have been shown to enhance plant growth by various mechanisms both under greenhouse and field conditions (Deepa et al., 2010; Panhwar et al., 2011; Mishra et al., 2011, Zahir et al., 2011; Yu et al., 2012; Estrada et al., 2013).

Among the notable PGPR, the organisms belonging to nitrogen fixing groups for example *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium* etc. have widely and traditionally been used over the years in well established agricultural practice for maintaining soil fertility which replenish nitrogen in the soil by forming symbiosis with legumes. During this interaction, the atmospheric N is converted to ammonia and other compounds and is hence, transported to growing plants. The effectiveness of this

strategy however, relies largely on maximizing symbiotic N_2 fixation (SNF) and plant yield to resupply organic and inorganic N and nutrients to the soil (Fox et al., 2007). Therefore, in order to increase the N pool of soils, rhizobial inoculants are commonly applied to soils/seeds of legumes to ensure effective nodulation and subsequent N_2 fixation (Dudeja and Singh 2008). However, there are also a few reports where rhizobia have been used in association with other free living rhizobacteria in order to achieve optimum legume production (Zaidi and Khan 2006; Mirza et al., 2007; Afzal et al., 2010). When used as mixture, the composite inoculant is likely to provide multiple benefits to the crop in addition to their normal individual activity. For instance, the synergistic effects of rhizobia and other free living PGPR have been found more effective than single inoculation and have tremendously increased crop production largely due to increased synthesis of phytohormones and nutrient absorption and mobilization, mainly P, N and C (Larrainzar et al., 2009; Yu et al., 2012; Cerqueira et al., 2013). Owing to the fact that nitrogen fixers enhance legume production across different ecological niches, it has become extremely important to find some novel and better performing rhizobia so that they could be developed as inoculants for ultimate transfer to field practitioners. Currently, numerous potential rhizobial species or biofertilizers in general with widely differing plant growth promoting activities have been recovered both from conventional (Zaidi et al., 2003 and 2004; Zaidi and Khan 2006; Khan and Zaidi 2007) and derelict soils (Wei and Ma 2011) and have been/are being marketed as biofertilizers/bioinoculants. Sadly, the impact of such fertilizers on crop production fluctuates from habitat (location) to habitat (location), plant genotypes to plant genotypes and from season to season depending on the survival of introduced microorganisms on seed, roots and in soil (Chanway and Holl 1992; Nowak 1998; Khalid et al., 2004; Hafeez et al., 2006). Therefore, in order to make effective use of such microbial inoculants, accurate and reliable methods for monitoring the fate of applied PGPR in the soil ecosystem are urgently needed so that their persistence in field environment and hence, their efficacy be improved.

Another major and most essential macro-elements is the phosphorus (P) which is required for growth and development of plants (including photosynthesis, energy and sugar production) and also promotes N_2 fixation in legumes (Saber et al., 2005). In soils, of the total P (0.5%), only 0.1% is plant available (Scheffer and Schachtschabel

1988). A greater part of P is present in the insoluble form and therefore, cannot be taken up by plants (Rodriguez and Fraga 1999). The deficiency of P in turn severely restricts growth and yields in plants. The problems of P deficiency are generally alleviated through the application of P fertilizers by field practitioners, whom they use to achieve maximum plant productivity; but this practice is expensive. However, the majority of P applied to soils is rapidly fixed into fractions that are poorly available to plant roots. Tropical and subtropical soils are predominantly acidic, and often extremely P deficient (Gaume 2000) with high P sorption (fixation) capacities. The concentration of soluble P in tropical soil is usually very low; available only in micromolar or lesser quantities (Goldstein 1994; Ozanne 1980). Inorganic P in acidic soils forms a complex with Fe and Al compounds (Norrish and Rosser 1983; Borling et al., 2001; Hao et al., 2002) while calcium phosphate predominates in neutral or calcareous soils (Sample et al., 1980; McLaghlin et al., 1988; Lindsay et al., 1989). Organic P also constitutes a large fraction of soluble P, as much as 50% in soils with high organic matter content (Barber 1984; Bishop et al., 1994; Oberson et al., 2001). Since the indiscriminate and excessive applications of chemical P fertilizers cause a profound adverse effect on the sustainability of crops and safety of the soil environment, agrarian communities are desperate to find alternative strategies that could ensure competitive yields while maintaining the nutrient pool of soils. Emphasis is therefore, being placed onto the possibility of greater utilization of unavailable P forms wherein the P-solubilizing microbes could play a pivotal role in making soluble P available to plants. Current developments in sustainability therefore, involve a rational exploitation of soil microbial activities and the use of less expensive, though less bioavailable, sources of plant nutrients, like rock phosphates (RP), which may be made available to plants by microbiologically mediated processes (Rajankar et al., 2007; Bojinova et al., 2008; Oliveira et al., 2009). Microorganisms involved in the solubilization of insoluble P include bacteria (Khan et al., 2010; Yasmin and Bano 2011; Oves et al., 2013), fungi (Khan et al., 2007; Khan et al., 2010) and actinomycetes (Balakrishna et al., 2012; Hamdali et al., 2012). Therefore, the use of microbial inoculants (biofertilizers) possessing P-solubilizing activities in crop productivity is considered as an environment-friendly alternative to further applications of mineral P fertilizers.

Apart from the major plant nutrients, other metabolites secreted by PGPR have been found to affect the health of plants. For instance, it is reported that during germination

of seeds the level of ethylene in the rhizosphere is greatly increased (Abeles et al., 1992). Also, a significantly higher level of ethylene has been reported in rhizobia inoculated legumes (Glick et al., 2007). The elevated level of ethylene in effect negatively regulates the nodulation process and diminishes the nodulation on inoculated plants (Middleton et al., 2007; Oldroyd and Downie 2008). The over-production of ethylene and its destructive effect on various physiological processes of plants including legumes have however, been found to be alleviated by the synthesis of ACC deaminase (Bhattacharjee et al., 2012). Different workers have proposed that some PGPR functions as a sink for aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene in higher plants, by hydrolyzing it to ammonia and α-ketobutyrate and in this way promote plant growth (Glick et al., 1998; Arshad et al., 2010). As an example, Shaharoona et al., (2006a) in a study reported that ACC deaminase containing *Pseudomonas* spp. improved the height, root weight and total biomass and significantly increased the yield of maize plants when grown under gnotobiotic conditions. In a follow up study, Noreen et al., (2012) observed a dramatic increase in the yield of *Pseudomonas* spp. inoculated mungbean (*Vigna mungo* L.) plants; notably *Pseudomonas* spp. had ACC-deaminase producing ability. Among nodule forming bacteria, that especially supply N to plants, strains of *Rhizobium* has also shown the production of ACC-deaminase and upon inoculation increased the nodulation, root weight, plant biomass and yield of pea and alfalfa (Ma et al., 2004; 2003). Similarly, *Mesorhizobium* carrying an exogenous ACC deaminase gene also improved the yield of chickpea (Nascimento et al., 2012a).

Pulses in general, are important source of dietary proteins and have the distinctive qualities of preserving and restoring nutrient deficient soils by forming symbiosis with nitrogen fixing rhizobia and hence, improve the physical properties and nutrient pool of soils. Pulse crops are reported to add a reasonable quantity of nitrogen (upto 30 Kg N/ha) to soils also. Of the different legumes grown around the world, chickpea (*Cicer arietinum*), pea (*Pisum sativum*), greengram (*Vigna radiata* L. wilczek) and lentil (*Lens esculentus*) serve as a rich source of protein in the Indian sub continent's dietary system and are popularly grown in many countries including Asian regions. However, the nutritional quality of such legumes varies greatly (Table 1).

Table- 1 Nutritional quality of different legumes used in the present study

Nutrient contents	Nutrient /100 g			
	Chickpea	Pea	Greengram	Lentil
Protein	19.3	23	23.9	24.2
Fat	6.1	2	1.1	2
Saturated fat	0.6	0.3	0.3	0.3
Carbohydrate	45.9	45.6	42.3	48.5
Dietary fibres	10.8	13	10	4.8
Insoluble fibre	7.5	9.4	7.2	3.5
Soluble fibre	3.3	3.6	2.8	1.3
Folate	0.557×10^{-3}	2.74×10^{-4}	6.25×10^{-4}	111×10^{-3}
Calcium	0.105	42×10^{-3}	132×10^{-3}	73×10^{-3}
Iron	0.006	3.8×10^{-3}	6.7×10^{-3}	7.5×10^{-3}
Magnesium	0.115	95×10^{-3}	189×10^{-3}	82×10^{-3}
Phosphorous	0.318	330×10^{-3}	367×10^{-3}	340×10^{-3}
Potassium	0.875	930×10^{-3}	1246×10^{-3}	840×10^{-3}
Zinc	0.0034	2.5×10^{-3}	2.7×10^{-3}	3×10^{-3}

Source: Chickpea and Mungbean- USDA National Nutrient Database (23. 2010); Pea and lentils- NUTTAB (2010)

India is the largest producer of pulse, accounting for about 25% of the global share. Presently, pulse production has remained around 13-15 million tonnes while annual domestic demand has increased to 18-19 million tonnes. According to the ministry of agriculture, India has however, achieved all time high record pulses production of 18.45 million tonnes (MT) in the 2012-2013 crop year ended June. The projected pulse requirement on the other hand by the year 2030 is estimated at about 32 million tonnes ICAR Vision 2030 (2011). Of the different pulses grown in different countries, chickpea in India occupies 7.7 million hectare and contributes about 50% of the total pulse production (Singh and Asthana 1999). Greengram widely grown in the tropical countries and in India, covers an area of three million-hectare, accounting for 14% of total pulses area and 7% of total production (Singh et al., 2004). Pea on the other hand is cultivated over an area of 5.9 million hectares with a production of about 11.7 million tones while in India, it is grown over an area of 0.7 million hectares accounting for about 0.6 million tones and contributes 3% and 5% to total area and pulse production, respectively ICAR Vision 2030 (2011). Lentil occupies 1.34 million ha and contributes 0.88 million tones to pulse production (ICAR 2006). Due to inadequate and conflicting reports on the performance of inoculated legumes in

different production systems and the possibility of damage to both PGPR and legumes due to the application of agrochemicals into the soils, it was desirable to explore the diversity of plant growth promoting rhizobacteria in terms of their functional variation. Subsequently, the effect of nitrogenous and phosphatic fertilizers and microbial inoculants on legumes popularly grown in this region was also investigated in order to find a novel and compatible bacterial pairing for developing efficient inoculants for enhancing legume production in different agro-ecological niches. To achieve these, the present investigation was therefore, designed with specific following objectives:-

(i) to assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area

(ii) isolation of nitrogen fixing bacteria from the nodules of legumes grown in conventional soils and phosphate solubilizing bacteria from different rhizospheric soils

(iii) to isolate ACC deaminase producing bacterial strains from different rhizospheres

(iv) to assay both qualitatively and quantitatively the production of plant growth promoting substances by the PGPR strains

(v) morphological, biochemical and 16S rRNA gene sequence based characterization of bacterial cultures and construction of phylogenetic tree

(vi) assessment of the impact of microbial inoculants on the performance of chickpea, pea, greengram and lentil grown in sandy clay loam soils treated with/without nitrogenous/phosphatic fertilizers and

(vii) nutrient uptake analysis in the test legumes.

2.1 Synthetic fertilizers and soil microorganisms

Synthetic fertilizers are widely used in agricultural practices particularly in developing countries to enhance soil fertility and hence, to crop production. Some argue that fertilizer was as important as seed in the Green Revolution (Tomich et al., 1995) contributing as much as 50% of the yield growth in Asia (FAO 1998; Hopper 1993). Others have found that one-third of the cereal production worldwide is due to the use of fertilizer and related factors of production (Bumb 1995). Fertilizer consumption in India has been increasing over the years and today India is one of the largest producer and consumer of fertilizers in the world. By 2009-10 total fertilizers consumption in India was 26.49 million nutrient tonnes (Jaga and Patel 2012). The importance/use of fertilizers in yield improvement is likely to increase further in order to achieve optimum agriculture production and consequently to feed the alarmingly increasing human populations because there is little scope for bringing more area under cultivation as well as majority of Indian soils are deficient in many macro and micro nutrients. However, the accumulation of such fertilizers in soils significantly affects biological and biochemical properties of soils (Marschner 2003; Yevdokimov et al., 2008; Zhong et al., 2010). Moreover, studies have mainly been conducted at a bulk soil scale or in short-term experiments, and as a result, there is still little available information on rhizosphere effects on extracellular enzyme activities and microbial community structure in agricultural soils as influenced by long-term practices. Among various factors, organic matter addition has been found to cause a rapid shift in the activities of various enzymes and reactivation of biogeochemical cycles in bulk soil (Madejon et al., 2001; Bastida et al., 2007). It is generally recognized that organic manure addition tends to increase the total microbial biomass, though the responses of specific groups such as Gram-positive bacteria, Gram-negative bacteria and fungi vary. For instance, organic manure additions often result in increased or altered fungal populations (Elfstrand et al., 2007; Bastida et al., 2007), altered populations of arbuscular mycorrhizal fungi (Corkidi et al., 2002), shifts in Gram-positive and Gram-negative bacteria (Peacock et al., 2001; Marschner 2003), and increased fungi/bacteria ratios (Elfstrand et al., 2007). Importantly, the response of the microbial community structure to organic manure additions tends to be based on differences in the carbon amount or quality of the organic amendments (Elfstrand et al., 2007). Inorganic fertilizers such as N, P and K have also been reported to affect the activities of soil enzymes (Goyal et al., 1999; Böhme et al., 2005). For example,

many hydrolytic enzyme activities of forest soil were increased by the addition of N fertilizer, but the phenol oxidase activity was dropped by 40% compared to control plots (Saiya-Cork et al., 2002). In yet other investigation, Weand et al., (2010) found that the N addition caused a change in the enzymatic activities in a soil which however depends on the nature of the dominant substrates (labile or recalcitrant). Furthermore, Phillips and Fahey (2008) found that rhizosphere effects on microbial activities and nutrient availability could be reduced by fertilizer addition in nutrient-poor forest soil, which they considered to be a result of fertilizer-induced shifts in the belowground C supply. Similarly, most studies have found obvious changes in soil microbial communities after addition of organic or inorganic fertilizer amendments (Peacock et al., 2001; Marschner 2003; Enwall et al., 2005). Changes in the soil microbial community structure are also observed after additions of inorganic N, P and K fertilizers (Zhang et al., 2007; Phillips and Fahey 2008; Yevdokimov et al., 2008). However, the ecological consequences of the application of various fertilizers in the rhizosphere are unclear, because of the poor understanding of how changes in nutrient availability impact on plant and soil microbial processes (Hobbie et al., 2002; Phillips and Fahey 2007). Fertilizer additions possibly result in decreased carbon allocation to roots and subsequent decreases in microbial respiration in the rhizosphere (Phillips and Fahey 2007). In another study, Buyer et al., (2010) reported that a vetch cover crop increased the amount and proportion of Gram-negative bacteria, fungi, and arbuscular mycorrhizal fungi in the rhizosphere of tomato plants.

2.2 Rhizosphere and root colonization

The rhizosphere is the narrow region of soil that is directly influenced by root secretions (Sørensen 1997) and associated soil microorganisms and plays some critical roles in plant growth and consequently the soil fertility (Rovira 1969). According to Bringhurst et al., (2001), the rhizosphere includes the region of soil bound by plant roots, often extending a few mm from the root surface. This region of soil is much richer in bacteria than the surrounding bulk soil (Hiltner 1904). In soil, microbes are often limited by energy and hence root exudates such as organic acids, sugars and amino acids provide energy to them and stimulate their growth and metabolic activities which in turn influence biogeochemical cycling of nutrients in soils (Cardoso and Freitas 1992; Stevenson and Cole 1999; Fontaine and Barot 2005).

Studies based on molecular techniques have estimated more than 4,000 microbial species per gram of soil (Montesinos 2003). Of these, about 10^7-10^9 colony forming units of culturable bacteria have been found in per gram of rhizosphere soil (Benizri et al., 2001) whereas the population densities in the rhizoplane has been reported to range from 10^5-10^7 colony forming units per gram of fresh weight (Benizri et al., 2001; Bais et al., 2006). Filamentous actinobacteria are also considered as one of the important community in rhizosphere microbiota (Benizri et al., 2001) being able to influence the plant development as well to protect the plant roots against phytopathogens. Changes in rhizobacterial community structure have been reported with the application of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) resulting in significant alterations in plant–microbes interactions (Herschkovitz et al., 2005). The PCR amplifications of 16S rDNA fragments were performed on an automated CR thermoblock (Eppendorf Mastercycler Gradient, Brinkmann Instruments, USA). PCR mixtures contained 5 µl of 10x magnesium-free buffer (Promega, Wisconsin, USA), 0.4 mg/ml bovine serum albumin, 3.75 mM $MgCl_2$, 300 mM of each deoxynucleoside triphosphate, 800 nM of each primer, 0.03 U μl^{-1} of Red Taq DNA polymerase (Sigma), and 1 µl of template DNA in a final volume of 50 µl using sterile 0.2 µm filtered double-distilled water. The PCR included denaturation at 95 ^{0}C for 1 min, 35 thermal cycles of 20 s at 95 ^{0}C, 25 s of annealing at 57 ^{0}C, and 30 s at 72 ^{0}C, ending with a 1-min extension step at 72 ^{0}C. Products were checked by electrophoresis in 1% (w/v) agarose and stained with ethidium bromide (0.2 mg/ml). The DGGE fingerprints was analyzed using the Dice algorithm (Dice coefficient of similarity) taking into account the presence of a band and its position when comparing samples for similarity. The unweighted pair group method with mathematical averages option was used for cluster analysis and for the construction of complete linkage dendrograms. Sequences were analyzed by CHIMERA_CHECK at the Ribosomal Database Project II (http:// rdp.cme.msu.edu/html) and suspected chimera sequences were removed from the analysis. Furthermore, the microbial populations first colonize the rhizosphere following soil inoculation (Gamalero et al., 2003) as shown by many techniques like, microscopic tools, immuno-markers or by fluorescence *in situ* hybridization (FISH) and by using gnotobiotic conditions. Following colonization, bacterial cells are visualized as single cells attached to the root surfaces, and subsequently as doublets on the rhizodermis, forming a string of bacteria (Hansen et al., 2000). From here onwards, the whole surface of some rhizodermal cells are colonized and bacteria can establish even as microcolonies or biofilms (Benizri et al., 2001). In a similar manner, rhizoplane colonization has been studied using both *in vitro* grown plants and plants grown in natural soil inhabiting a high microbial

diversity. In order to provide benefits to plants, such microorganisms (inoculated one/natural inhabitants of soils) thus must be rhizosphere and/or rhizoplane competent (Elliot and Lynch 1984; Compant et al., 2005) for an extended period of times (Whipps 2001). Many factors can be involved in rhizosphere and rhizoplane competence by PGPB. However, the competence of bacteria varies among different rhizospheres/rhizoplane Gamalero et al., (2003) which has been described to be linked to root exudation (Lugtenberg and Dekkers 2001). For instance, carbon fixed by plant photosynthesis is known to be partly translocated into the root zone and released as root exudates (Bais et al., 2006). Moreover, various carbohydrates, amino acids, organic acids, and other compounds, which provide a source of nutrients for root-associated bacteria, are released in the rhizosphere (Walker et al., 2003). Such exudates act as chemo-attractants towards which the bacterial population moves, and in effect allow them to colonize and multiply both in the rhizosphere and the rhizoplane (Lugtenberg and Kamilova 2009). Plant exudates thus provide a rich source of energy and nutrients for the bacteria in rhizosphere, resulting in more microbial populations in the region than outside the region (Haas and Defago 2005). The colonization of plant rhizosphere by *Azospirillum* sp., *Bacillus subtilis* sp., and *Pseudomonas* sp., has been well studied (Steenhoudt and Vanderleyden 2000; Trivedi et al. 2005). Rhizobacteria may depend on other microbes for nutrient sources as one microbe may convert plant exudates into a form that can be used by another microbe. Thus, rhizosphere has appeared as a versatile and dynamic ecological environment of intense plant-microbe interactions (Mayak et al., 2004a) harnessing essential micro and macro-nutrients affecting plant growth, although, the process of root colonization is under the influence of various parameters such as bacterial traits, root exudates and several other biotic and abiotic factors (Benizri et al., 2002). Broadly, chemotaxis is generally considered to play an important role for successful rhizosphere/rhizoplane colonization (Andrews and Harris 2000; Walsh et al., 2001). Recently, it has been reported that soil microorganisms, including free-living as well as associative and symbiotic rhizobacteria belonging to the genera like *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Xanthomonas* in particular, are the integral parts of rhizosphere biota (Kaymak 2011; Glick 1995) exhibiting successful rhizosphere colonization. Lugtenberg et al., (2001) reported a large number of cell surface molecules as responsible for the effective rhizosphere

responsible for the effective rhizosphere colonization. Rhizospheric colonization is thus, considered as a crucial step in the application of microorganisms for beneficial purposes such as biofertilization, phytostimulation, biocontrol and phytoremediation, although the colonization of rhizosphere by PGPRs is not a uniform process. For example, *Kluyvera ascorbata* colonized the upper two-thirds of the surface of canola roots but no bacteria were detected around the root tips (Ma et al., 2001).

2.3 Pulse production: A brief account

Pulses are the second most important nutritional group of crops after cereals in the dietary system of many countries. India is the largest producer and consumer of pulses in the world accounting for about 25% of global production, 27% of consumption, and 34% of food use (FAO 2009). According to the Indian Council of Agricultural Research (ICAR), an apex body of the National Agricultural Research System, Ministry of Agriculture, Government of India, pulses production in India has been hovering around 13-15 million tonnes during the last decade, while annual domestic demand has risen to 18-19 million tonnes. During 2010-11, the production of pulses in India, estimated at 17.29 million tonnes, was an all-time high record. According to the ministry of agriculture, the country has however, achieved all time high record pulses production of 18.45 million tonnes (MT) in the 2012-2013 crop year ended June. The previous pulses production record was 14.91 million tonnes during the year 2003-2004. Among kharif pulses (7.3 million tonnes), pigeonpea (3.15 million tonnes) and blackgram (1.82 million tonnes) production are slated to hit all time higher. The all time high production record of 18.45 miilion tonnes could be possible primarily due to availability of quality seeds to pulse growers. Apart from availability of quality seeds of high yielding varieties, the strong technology back-up, favourable monsoon, increase in minimum support prices and effective government programmes helped for increasing production of pulses in the country. The projected pulse requirement by the year 2030 is estimated at about 32 million tonnes ICAR Vision 2030 (2011). In India, about dozen of pulse crops, namely chickpea, pigeonpea, mungbean, urdbean, lentil, field pea, lathyrus, cowpea, common bean, moth bean, horsegram and ricebean are cultivated on 22.47 million ha area under varied agro-ecological conditions. About 90% of the global pigeonpea, 75% of chickpea and 37% of lentil area falls in India (FAOSTAT 2009). Globally, the pulse production in 2009 was 61.5 million tons over an area of 70.6 million ha with an average yield of 871 kg/ha. Of these, beans contributed about 32% to global pulse production which was followed by dry peas

(17%), chickpea (15.9%), broad beans (7.5%), lentils (5.7%), cowpeas (6%) and pigeonpea (4%). Among different nations, developing countries contribute about 74% to the global pulse production and the remaining comes from developed countries. India, China, Brazil, Canada, Myanmar and Australia are the major pulse producing countries with relative share of 25, 10, 5, 5 and 4%, respectively. Countries recording annual production growth of more than 4% are Myanmar (11.48%), Canada (10.80%), Germany (8.27%), Sudan (8.08%), Spain (7.37%), Ethiopia (4.92%), China (4.67%) and Syria (4.12%) presented in ICAR vision 2030 (2011).

2.4 Rhizobium–legume symbiosis: An overview

The microbiological process that converts atmospheric dinitrogen (N_2) into a plant-accessible species of N is generally known as biological nitrogen fixation (BNF). Through BNF, the external application of chemical N fertilizers in different agronomic practices can be reduced, if not completely abolished (Peoples et al., 1995a; Herridge et al., 2008). Total global N_2 fixation from BNF has been estimated to 100–290 million tonnes N $year^{-1}$ with approximately 50–70 million tonnes N $year^{-1}$ in agricultural systems, compared with 83 million tonnes N fixed industrially in fertilizer production. The symbiotic systems are however, a major source of N for most legume crops with an average of 80% of N derived from BNF (Vance 2001; Graham and Vance 2003). There are estimates that the rhizobial symbioses with 18,000 legume species (Masson-Boivin et al., 2009) including more than 100 agriculturally important legumes spanning all the geographical regions contribute nearly half of the annual quantity of BNF in soil ecosystems (Graham and Vance 2003). Rotations of legumes with other non-nitrogen fixing plants enrich the soil with fixed N and increase the productivity and sustainability of agricultural systems. There is evidence that N derived from legume sources are less susceptible to losses than chemical fertilizer N, which in long term results in the build-up of a reserve of readily mineralizable organic nitrogen.

Among the wide array of bacteria that have the ability to reduce atmospheric N to usable forms of N, the most notable are the rhizobial species that forms a strong and viable symbiotic relationship with leguminous plants (Table 2) belonging to β-proteobacteria of the genera *Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* (*Ensifer*), collectively called rhizobia (Perret et al., 2000; Jones et al., 2007; Franche et al., 2009). Recently, several new species of N_2-fixing microsymbionts, such as, *Methylobacterium* (Sy et al., 2001), *Herbaspirillum*

(Valverde et al., 2003), *Ochrobacterium* (Zurdo-Pineiro et al., 2007), *Phyllobacterium* (Valverde et al., 2005), and members of the β-proteobacteria such as *Burkholderia* (Moulin et al., 2001) and *Cupriavidus* (Ralstonia) (Chen et al., 2001) have been discovered. A successful interaction between legume plants and rhizobia leads to the formation of nodules on the roots or shoots. Bacteria in the form of bacteroids reside inside nodules and fix atmospheric N into ammonia (Perret et al., 2000; Gibson et al., 2008). The reduced nitrogenous compounds are then transported into the host plant in exchange for organic acids. In the *Rhizobium*–legume symbiosis, the rhizobium are housed inside a novel organ, the root nodule. The formation of this organ, through the reprogramming of root cortical cells, is set in motion by specific lipochito-oligosaccharides called Nod factors that are secreted by rhizobia (Oldroyd and Robatzek 2011). At the same time, Nod factors control the formation of tubular, transcellular, cell wall-bound infection structures, called infection threads. In most of the advanced legumes, infection threads originate in root hairs and guide the bacteria to nodule primordium cells that are formed from reprogrammed root cortical cells (Oldroyd and Robatzek 2011). There, the bacteria are released from the infection threads into the developing nodule cells. However, the processes of host-microbe signalling and colonization and the mechanisms leading to mutual benefits are less-well characterized. Though, attempts to know the molecular ecology and interactions are underway, a high amount of progress is required to fully understand the mechanism of establishment, the way interactions take place in plant, between different microbes and plants and exclusive benefits by endophytes and plants (Dudeja et al., 2012). Despite their key importance, the molecular and cellular mechanisms underlying the formation of these membrane interfaces are largely unknown. Ivanov et al., (2012) showed that the two highly homologous exocytotic vesicle-associated membrane proteins (VAMPs) are required for the formation of symbiotic membrane interface in both interactions. Further, silencing of these VAMPs genes had a minor effect on non-symbiotic plant development and nodule formation. However, it blocks symbiosome and arbuscule formation, whereas root colonization by the microbes is not affected. Identification of these VAMPs as common symbiotic regulators in exocytotic vesicle trafficking suggests that the ancient exocytotic pathway forming the peri-arbuscular membrane compartment have co-opted in the *Rhizobium*-legume symbiosis. Some of them, such as *Rhizobium* sp. NGR234, are extremely promiscuous and are able to nodulate many different host plants, over 112

hosts (Pueppke and Broughton 1999), while others, such as *R. leguminosarum* bv. *trifolii* have a very narrow host range and nodulates only clover (*Trifolium* sp.) plants. Its close relative, *R. leguminosarum* bv. *viciae*, nodulates pea, vetch (*Vicia* spp.), lentil and sweet pea (*Lathyrus* spp.) (Perret et al., 2000). In the early steps of symbiosis, a diverse array of compounds is exuded into the rhizosphere, including flavonoids, isoflavonoids and non-flavonoid inducers. These compounds act as chemo-attractants for rhizobia (Dharmatilake and Bauer 1992; Cooper 2007), influence bacterial growth and induce the expression of nodulation genes (nod genes) (Hungria and Stacey 1997). As a result of nod genes expression, biosynthesis of specific lipochitin oligosaccharides called nodulation factors (Nod factors or LCOs) occurs (Lerouge et al., 1990). Nod factors are structurally diverse and a single rhizobial strain may produce a range of these metabolites (Spaink et al.,1991; 1995).

Table 2- Current information on available rhizobial species

Genus	No. of species	Major host plants
Rhizobium	33	*Pisum, Phaseolus* etc.
Sinorhizobium	12	*Acacia, Medicago* etc.
Mesorhizobium	19	*Cicer, Prosopis* etc.
Bradyrhizobium etc.	8	*Glycine, Pachyrhizus*
Azorhizobium	2	*Sesbania*

Compiled from: Rivas et al., (2009)

2.5 Plant Growth-Promoting Rhizobacteria: mechanism of action and growth promotion

Plant growth promoting rhizobacteria affects the plant growth both indirectly and directly (Glick et al., 1999; Antoun and Pre'vost 2006). Of these, the indirect promotion of plant growth occurs when PGPR reduces or prevent the deleterious effects of one or more phytopathogenic organisms by-(i) synthesizing antibiotics (Burd et al., 2000; Glick 2001) (ii) depleting the availability of iron in the rhizosphere (Solano et al., 2010) (iii) inducing systemic resistance (Choudhary and Johri 2009) (iv) synthesizing antifungal metabolites for example production of fungal cell wall lysing enzymes (Chen et al., 2010) (v) competition (Hofte and Altier, 2010) (vi) stimulating beneficial symbioses (Yu et al., 2012; Glick 2014) and (viii) by decreasing the toxicity of hazardous substances in contaminated soils (Ahemad and Khan 2012; Oves et al., 2013; Wani and Khan 2013). On the contrary, the direct mechanisms of plant growth promotion by PGPR involves the (i) N_2 fixation (Wani et al., 2007c) (ii)

solubilization of insoluble phosphorus (Khan et al., 2006, Khan et al., 2009; Khan al., 2010) (iii) sequestering of iron by production of siderophores (Rajkumar et al., 2006; Wani et al., 2008a) (iv) production of phytohormones such as, auxins, cytokinins, gibberellins (Wani et al., 2007a, b; Ahmad et al., 2008b), and (v) lowering of ethylene concentration (Glick et al., 2007; Rodrigues et al., 2008; Wani et al., 2008a) (v) synthesis of compounds by the bacterium or PGPR facilitates the uptake of certain nutrients from the environment (Azcon 1989) and to make these elements accessible to plants (Perveen et al., 2002; Khan and Zaidi 2007; Wani et al., 2007a, b) Summarily, the PGPR functions in three different ways- (i) synthesizing particular compounds for uptake by plants (Khan et al., 2007; Zaidi and Khan 2006; Zaidi et al., 2003) (ii) facilitating the uptake of certain nutrients from environment (Garcıa et al., 2001; Cakmakci et al., 2006) and (iii) protecting plants from diseases (Trivedi et al., 2008; Guo et al., 2004; Pandey et al., 2006). Apart from the different mechanisms of plant growth promotion, PGPR in order to provide benefits directly or indirectly to plant must be able survive in the rhizospheres, colonize the root surfaces and multiply after colonization. An overview of the plant growth promotion by PGPR is presented in (Fig 1) while the growth-promoting substances synthesized by various PGPR are summarized in (Table 3). Among many PGPR inhabiting soil, the SNF enhance the growth of legumes by- (i) BNF (ii) increasing the availability of nutrients in the rhizosphere (iii) inducing increases in root surface area (iv) enhancing other beneficial symbioses of the host (v) reducing or preventing the deleterious effects of phytopathogenic organisms (Khan et al., 2002) and (6) combination of modes of action. For instance, IAA produced by many rhizobia (Abd-Alla 1994; Wani et al., 2007a, 2007b, 2008a, 2008b; Ahemad and Khan 2012; Oves et al., 2013), and its metabolically related precursor, anthranilic acid, can reductively solubilize soil Fe (III), and increase its availability via a mechanism different from that involving siderophores.

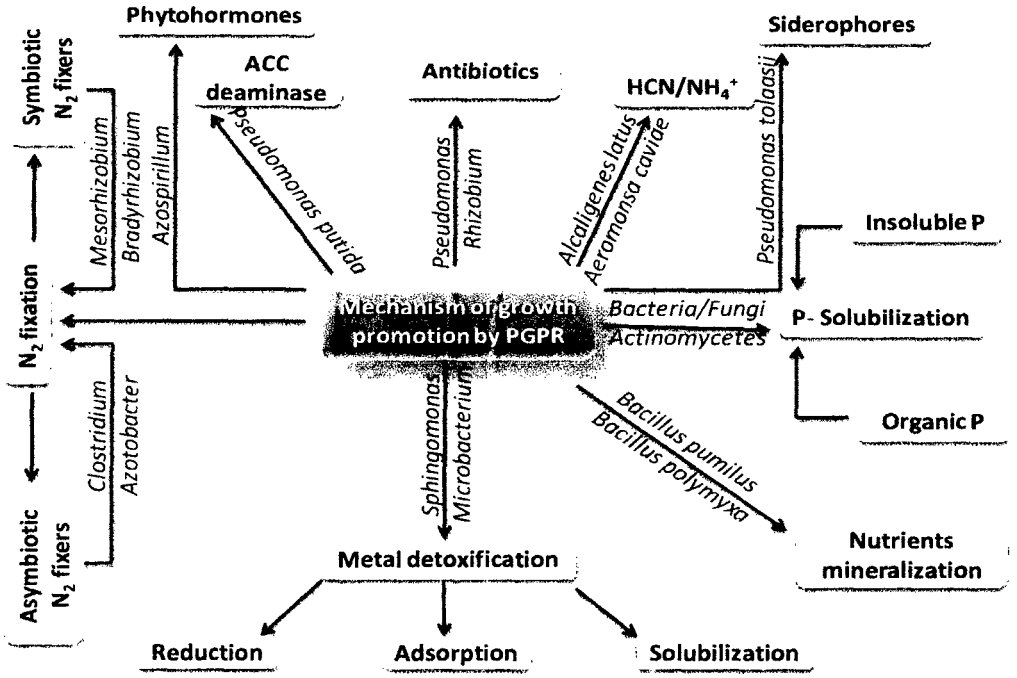

Fig. 1 A general scheme showing how PGPR promote plant growth (adapted from Khan et al., 2009)

Table 3- Growth promoting substances produced by plant growth promoting rhizobacteria

Organisms	Growth regulators	Reference
Pseudomonas sp., *Pseudomonas fluorescens*, *Burkholderia glumae*	ACC deaminase, IAA, Siderophore, Ammonia, HCN, P-solubilization	Rashid et al., (2012)
Bacillus	ACC deaminase, IAA, Siderophore, P-solubilization, Lytic enzyme, HCN	Kumar et al., (2012)
Azotobacter	IAA, Siderophore, P-solubilzation	Farajzadeh et al., (2012)
Pseudomonas	IAA, Siderophore, P-solubilzation, HCN	Ahemad and Khan (2011)
Azotobacter, Fluorescent *Pseudomonas*, and *Bacillus*	IAA, Siderophore, Ammonia, HCN, P-solubilization	Ahmad et al., (2008)
Pantoea dispersa strain 1A	P solubilization, IAA, Siderophore, HCN	Selvakumar et al., (2008)
Bacillus spp.	IAA, siderophore, HCN	Wani et al., (2007c)
Pseudomonas, *Bacillus*	Siderophore, IAA, P-solubilization	Rajkumar et al., (2006)
Brevibacillus sp.	IAA	Vivas et al., (2006)
Xanthomonas sp. RJ3, *Azomonas*	IAA	Sheng and Xia (2006)

sp. RJ4, *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ31		
Bacillus sp.	P-solubilization	Canbolat et al., (2006)
Brevibacterium sp.	Siderophore	Noordman et al., (2006)
Bacillus subtilis	IAA and P-solubilization	Zaidi et al. (2006)
Variovorax paradoxus, *Rhodococcus* sp. and *Flavobacterium* (Cd tolerant)	IAA and Siderophore	Belimov et al., (2005)
Pseudomonas fluorescens	IAA, siderophore and P-solubilization	Gupta et al., (2005)
Pseudomonas putida	Siderophore	Tripathi et al., (2005)
Azotobacter, Fluorescent *Pseudomonas*	IAA	Ahmad et al., (2005)
Bacillus and *Azospirillum* sp.	IAA, P-solubilization	Yasmin et al., (2004)
Pseudomonas aeruginosa	IAA, Siderophore, HCN	Bano et al., (2003)
Bacillus, *Pseudomonas*, *Azotobacter*, and *Azospirillum*	P-solubilization and IAA	Tank and Saraf (2003)
Pseudomonas sp.	Siderophore	Sharma et al., (2003)
Pseudomonas sp.	IAA, Siderophore and P-solubilization	Gupta et al., (2002)
Pseudomonas fluorescens	Siderophore	Khan et al., (2002)
Azotobacter chroococcum	Gibberellin, Kinetin, IAA,	Verma et al., (2001)
Kluyvera ascorbata	Siderophore	Burd et al., (2000)

Table 4- Table: 2 Examples of plant growth-promoting substances synthesized by symbiotic nitrogen fixers

Symbiotic N_2 Fixer	Crop Enhancer	References
Bradyrhizobium MRM6	IAA, HCN, Siderophore, Ammonia, EPS	Ahemad and Khan (2011c)
Rhizobium MRL3	IAA, HCN, Siderophore, Ammonia	Ahemad and Khan (2011d)
Sinorhizobium strain	Chitinase	Qing-xia et al., (2011)
Rhizobium leguminosarum var. *phaseoli*	IAA	Stajkovic et al., (2011)

Rhizobium spp.	IAA, Siderophore	Mehboob et al., (2011)
Sinorhizobium meliloti	IAA, P-solubilization	Bianco et al., (2010)
Bradyrhizobium	IAA, Gibberellic acid	Afzal et al., (2010)
Mesorhizobium	IAA	Ahemad and Khan (2010a)
Rhizobium spp.	IAA	Chakrabarti et al., (2010)
Rhizobium leguminosarum	IAA, Siderophore	Ahemad and Khan (2010c)
Mesorhizobium	IAA, HCN, Siderophore, Ammonia, P-solubilization	Ahmad et al (2008)
Rhizobium strain TAL 1145	ACC-deaminase	Tittabutr et al., (2008)
Rhizobium spp,	IAA, Gibberellic acid, Zeatin	Boiero et al., (2007)
Mesorhizobium loti MP6	IAA, HCN, Siderophore, P-solubilization	Chandra et al., (2007)
Rhizobium etli USDA9032	Pheenazine, Antibiotic	Krishnan et al., (2007)

Adapted from Ahmad et al., (2012)

2.5.1 Some examples of positive plant growth regulators

Plant growth regulators (PGRs) are the substances that influence physiological processes of plant at very low concentrations and modify or control one or more specific metabolic events of a plant (Danova et al., 2012; Sane et al., 2012). According to the Environmental Protection Agency (EPA), the plant regulators have been defined as "any substance or mixture of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise alter the behaviour of plants or their produce." Such compounds produced by the plant or by PGPR are called plant hormones (Davies 1995; Karadeniz et al., 2006). Broadly, on the basis of chemical structures and their subsequent effects on plants, plant growth regulating substances have been divided into five general groups- (i) auxins (ii) gibberellins (iii) cytokinins (iv) ethylene and (v) a group called inhibitors, which includes abscisic acid (ABA), phenolics, and alkaloids (Frankenberger and Arshad 1995; Ferguson and Lessenger 2006). The production of auxins (Glick 1995; Wani et al., 2007a; Wani et al., 2008; Ahemad and Khan 2012) and ethylene (Sasek et al., 2012) for example by PGPR is considered a common

microbiological trait while the synthesis of cytokinins is less common. The gibberellin secretion at high concentrations is however, very rare (Solano et al., 2008). Generally, majority (>80%) of the soil bacteria are capable of secreting auxins especially IAA, indole butyric acid or similar compounds via tryptophan metabolism (Solano et al., 2008; Legault et al., 2011). A few examples of the phytohormones secreted by PGPR (Table 3) including rhizobia (Table 4) and other compounds and their direct or indirect impact on plant growth and development is reviewed and discussed briefly in the following section.

2.5.1.1 Phytohormones: Importance in plant growth

The production of phytohormones such as auxins, cytokinins and gibberellins by natural soil microbial communities have been reported by various workers over the last 20 years (Giordano et al., 1999a, Giordano et al., 1999b; Poonguzhali et al., 2008; Rajkumar and Freitas, 2008; Selvakumar et al., 2008; Singh et al., 2008; Ahemad and Khan 2012). Among the variously distributed plant hormones, auxins are the major plant growth regulators that stimulate cell division and elongation and production of auxin by PGPR is one of the most widely studied and, perhaps, the most effective mechanism of plant growth promotion by PGPR (Pereira et al., 2006; Schlindwein et al., 2008). Once released outside, the IAA is known to control many important physiological processes (Fig 2). Inside plant cells, IAA is largely formed by *de novo* synthesis from tryptophan, which undergoes either oxidative deamination (via the formation of indole-3-pyruvic acid) or decarboxylation (via the formation of tryptamine, with indole-3-acetic aldehyde as an intermediate. The synthesis of IAA by microbes involves one of the three pathways as presented in Fig 3 (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in the majority of bacteria like, *Erwinia herbicola*; saprophytic species of the genera *Agrobacterium* and *Pseudomonas*; certain representatives of *Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella*, and *Enterobacter* (2) The conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway in which tryptamine is formed. This pathway is believed to operate in pseudomonads and azospirilla and (3) IAA biosynthesis via indole-3- acetamide formation is reported for phytopathogenic bacteria *Agrobacterium tumefaciens, Pseudomonas syringae*, and *E. herbicola*; saprophytic pseudomonads like (e.g. *Pseudomonas putida* and *P. fluorescens*). The genes controlling IAA synthesis via this pathway are also reported in symbiotic bacteria like, *Rhizobium* spp., *Bradyrhizobium* spp., and *Azospirillum* spp., although

the activity of the corresponding enzymes is either negligible or not detectable. (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in plants, *Alcaligenes faecalis*, and possibly the cyanobacterium (*Synechocystis* sp.) and (5) The tryptophan-independent pathway, more common in plants, is also found in microorganisms (azospirilla and cyanobacteria). However, the synthesis of IAA using this pathway is reported to be insignificant, and the mechanisms are largely unknown. Many bacteria are known to synthesize auxins using such pathways and help the plants to grow better. Bacteria in general forms maximum amount of IAA during the steady-state stage of their growth while ammonium ions and glutamine inhibit IAA biosynthesis (Tsavkelova et al., 2006). The genes involved in IAA synthesis in bacterial strains may be plasmid or chromosomal borne. For example, pathogenic bacteria contain Ti plasmids that control the formation of the phytohormone, whereas in saprophytic microorganisms, auxin biosynthesis is governed by chromosomal genes (Tsavkelova et al., 2006). It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Loper and Schroth 1986). Of the various PGPR strains, bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes*, *Enterobacter*, *Acetobacter* and *Bradyrhizobium* have been shown to produce auxins which help in stimulating plant growth (Egamberdieva et al., 2007; Wani et al., 2007a; Kumar et al., 2008; Poonguzhali et al., 2008). However, the extent of IAA production by bacterial strains could be different due in part to the involvement of biosynthetic pathways, location of the genes, regulatory sequences, and the presence of enzymes to convert active free IAA into conjugated forms. Moreover, the synthesis of IAA is also influenced by environmental factors (Patten and Glick 1996). Synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has also been demonstrated (Wani et al., 2007b). In a similar study, Bent et al., (2001) reported that the concentration of indole compounds by three different strains, *Paenibacillus polymyxa* (L6), *P. poly*myxa (Pw-2), and *Pseudomonas fluorescens* (M20) increased with increasing rate of tryptophan (0-200 mg/ml) at different incubation interval.

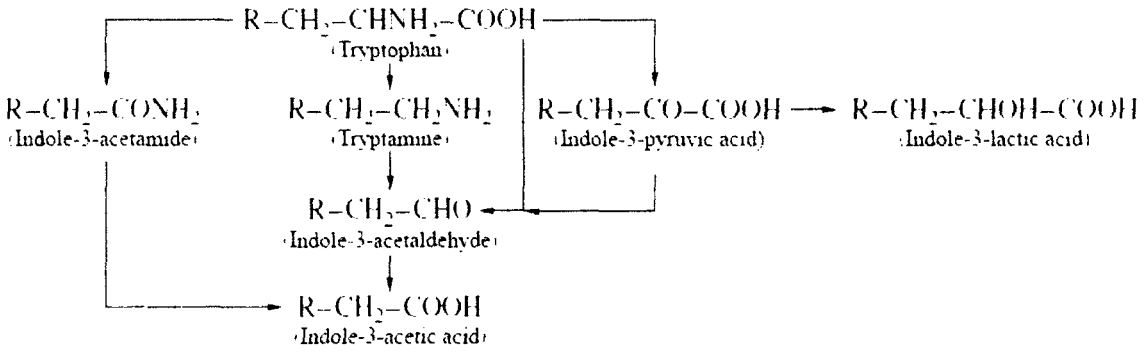

Fig. 2 Biosynthetic pathways of IAA synthesis in bacteria (Adapted from Patten and Glick 1996)

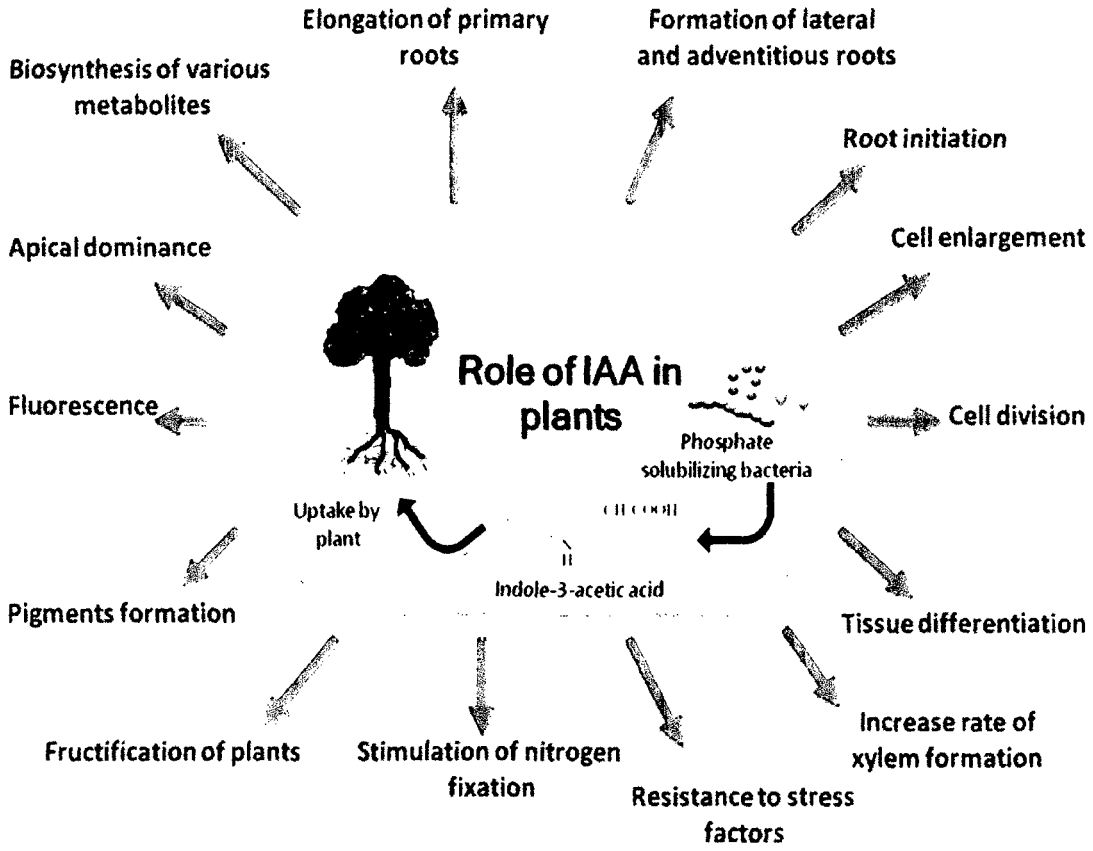

Fig. 3: Indole acetic acid affecting various stages of plant development (Adapted from Ahemad and Khan 2009)

2.5.1.2 Role of Indole Acetic acid in legume-*Rhizobium* symbiosis

Among nodule bacteria, rhizobial strains have been reported to produce auxins in variable amounts. For example, Antoun et al., (1998) working with 266 rhizobial strains belonging to different genera found that 58% of the strains produced IAA, while Vargas et al., (2009) in a similar study reported considerably lower frequency of auxin producers (23%) among populations of clover nodulating *R. leguminosarum* bv. *trifolii*. The auxins so released by nodule bacteria is reported to affect nodulation,

and accordingly IAA synthesizing rhizobia have been found to produce more nodules than IAA negative mutants (Boiero et al., 2007). The IAA produced by rhizobia may also induce root morphogenesis and consequently enhance its- (i) size and weight (ii) branch numbers and patterns and (iii) the surface area of roots as reported in non-legumes (Dazzo and Yanni 2006). Inoculation with auxin-producing bacteria may also result in the formation of adventitious roots (Solano et al., 2008). Furthermore, Noel et al., (1996) observed that the inoculation with IAA producing strains of *R. leguminosarum* accelerated the germination of canola and lettuce. Similarly, Biswas et al., (2000) concluded that the inoculation of rice with *R. leguminosarum* bv. *trifolii* increased dry matter and grain production, besides an increment in N, P, K, and Fe content in plant tissue. All these effects were ascribed due to the accumulation of IAA in the rhizosphere following rhizobial inoculation leading to some physiological changes in the root systems with consequent increase in nutrient uptake. In contrast, the over production of IAA in some cases by PGPR has been found to have deleterious impact on to plants (Schlindwein et al., 2008). For example, *R. leguminosarum* bv. *trifolii* strain TV-13 produced 171.1 mg/ml IAA in media enriched with tryptophan (Schlindwein et al., 2008), while strains of *Bradyrhizobium* sp. isolated from black wattle roots produced between 1.2 and 3.3 mg/ml IAA and increased the seedling vigor in relation to un-inoculated control plants. The variation in the amount of IAA produced by PGPR was however, suggested due to differences in the composition of the growth medium and tryptophan-concentration. In a follow up study, Sridevi et al., (2008) observed that IAA production by rhizobia occurred only when tryptophan was added to YM and that the isolates produced the maximum amount of IAA in medium supplemented with 2.5 mg/ml tryptophan concentration.

2.5.1.3 Other phytohormones

Like auxins, cytokinins affects both cell division and cell enlargement and also affects seed dormancy, flowering, fruiting, and plant senescence (Ferguson and Lessenger 2006). Cytokinins production by PGPR (Boiero et al., 2007) is however, less obvious compared to the production of auxins. This is probably due to the lack of methods used for cytokinins detection and hence, reports on cytokinin synthesis by PGPR in general are scarce. Gibberellin is yet another growth regulators which affects-(i) seed germination (Miransari and Smith 2009) (ii) stimulate growth of plants (Gou et al., 2011) and (iii) delay aging (Ferguson and Lessenger 2006). The production of gibberellins at high concentrations is considered very rare and has been reported for

two strains of *Bacillus,* isolated from the *Alnus glutinosa* rhizosphere (Solano et al., 2008). The concentration of gibberellins in nodules is, however, generally higher than in nearby root tissue as supported by the fact that rhizobia have the capacity to produce some amount of gibberellin-like substances. However, it is not known whether bacteria contribute significantly to the amount of gibberellins within the nodule or it is just imported from some remote host plant tissue (Dobert et al., 1992; Hedden and Thomas 2012). Despite all these contrasting facts, the role of gibberellin in *Rhizobium*–legume symbiosis that may have important implications in the endophytic colonization of non-legumes by rhizobia is adequately described. For example, *A. caulinodans* infects the semi aquatic legume *Sesbania rostrata* via the intercellular crack entry, a process mediated by gibberellins. Considering that crack entry is the main process of endophytic colonization of non-legumes by rhizobia, the production of gibberellins by the bacterium is reported to facilitate this process (Lievens et al., 2005).

2.5.1.4 Negative plant growth regulator

Abscisic acid is one of the strong inhibitor of growth and germination and promotes seed dormancy (Miransari and Smith 2009; Yang et al., 2009). Apart from these, ABA also helps plants to tolerate abiotic stresses. When plants are exposed to drought stress the hormonal balance of plants change and increasing ABA content in the leaves increases, which reduce the level of cytokinin. This in turn elicits stomata closure (Yang et al., 2009). Cohen et al., (2009) in a similar study suggested that ABA produced along with gibberellins by PGPR strain, significantly contributed to water-stress alleviation of maize plants. Some rhizobial strains such as *B. japonicum* USDA110 also produce ABA (Boiero et al., 2007) and functions in the same way as do the other PGPR (Zheng et al., 2012).

2.5.1.5 Growth modulation enzyme ACC deaminase: An overview

Ethylene is a plant hormone that is involved in the regulation of many physiological processes, such as leaf senescence, leaf abscission, epinasty, and fruit ripening (Arshad and Frankenberger 2002). Also, ethylene regulates nod factor signaling and nodule formation and has primary functions in plant defense systems. Besides its physiological role in different developmental stages of plants, ethylene is also considered as a stress hormone, whose synthesis in plants is increased substantially by a number of biotic and abiotic stresses. At higher concentrations, ethylene, however, inhibits growth and development of plants (Grichko and Glick 2001). The enzyme 1-

aminocyclopropane-1-carboxylate (ACC) deaminase which however circumvent the ethylene stress was first purified to homogeneity from *Pseudomonas* sp. strain ACP (Honma and Shimomura 1978), was subsequently partially purified from *Pseudomonas chloroaphis* 6G5 (Klee et al., 1991) and *Pseudomonas putida* GR12-2 (Jacobsonet al., 1994) and then purified to homogeneity from *P. putida* UW4 (Hontzeas et al., 2004). Later on ACC deaminase has been found to be synthesized by variety of PGPR (Belimov et al., 2005; Safronova et al., 2006; Rajkumar et al., 2006; Madhaiyan et al., 2006; Mellado et al., 2007) and strongly alleviates the stress induced by ethylene-mediated impact on plants by hydrolyzing ACC, the immediate precursor of ethylene in plants to NH_3 and a-ketobutyrate (Honma and Shimomura 1978; Glick et al., 1998; Penrose and Glick 2001; Reed et al., 2005; Safronova et al., 2006), and consequently reduce the ethylene levels in plants (Glick et al., 1998, 2007), The bacteria utilize the NH_3 evolved from ACC as a source of N and thereby restrict the accumulation of ethylene within the plant, which otherwise inhibits plant growth (Belimov et al., 2002). Thus, the decreased levels of ethylene in turn allow the plants to grow better (Madhaiyan et al., 2007; Zahir et al., 2008). It has been observed that plants that are inoculated with PGPR containing ACC deaminase are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as flooding (Grichko and Glick 2001), heavy metals (Burd et al., 1998; Grichko et al., 2000), presence of phytopathogens (Wang et al., 2000), drought, and high salt contents (Mayak et al., 2004). In most of these cases, it has been reported that the PGPR-containing ACC deaminase significantly lowered the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence damage to the plants.

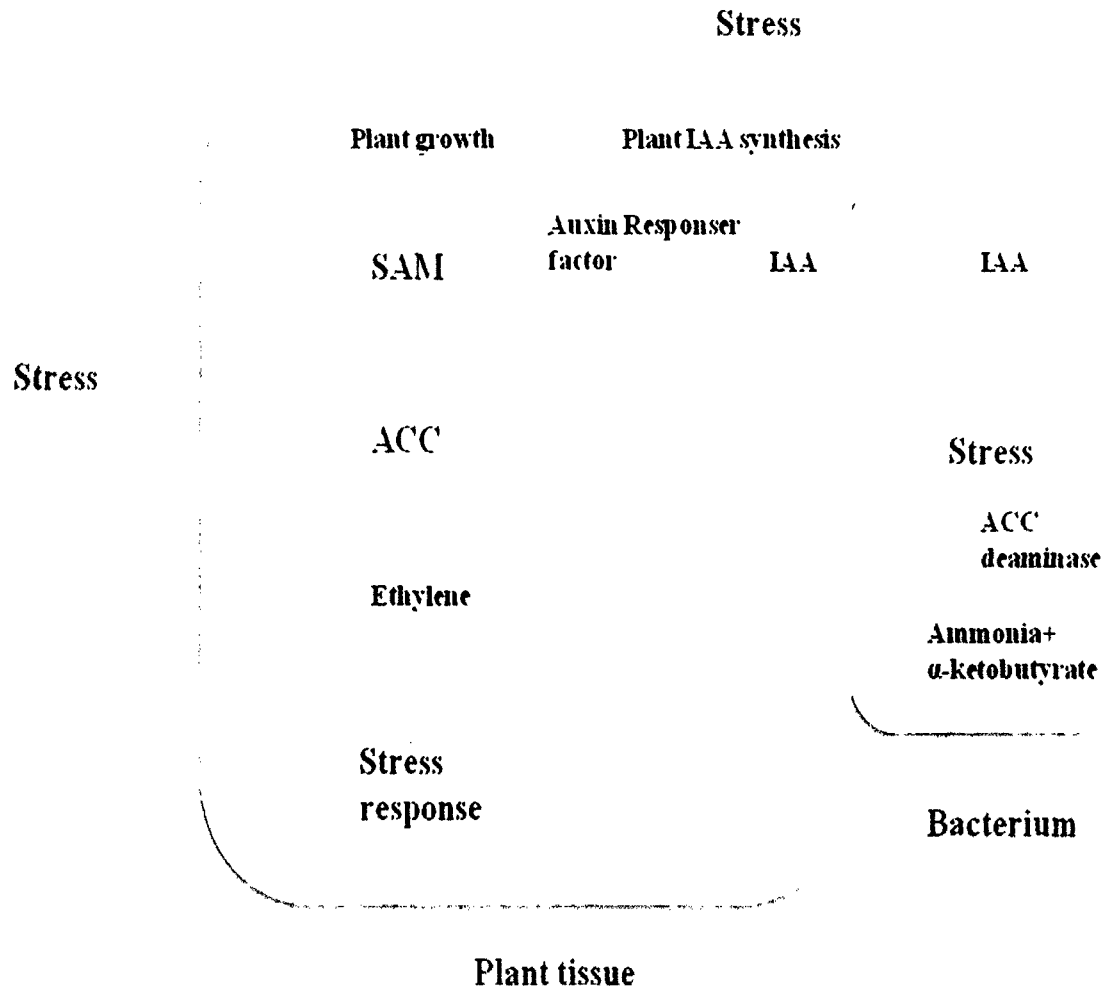

Fig. 4 A schematic model of how plant growth-promoting bacteria that both pro-duce ACC deaminase and synthesize IAA may facilitate plant growth. The only enzyme shown in this scheme is ACC deaminase. SAM is converted to ACC by the enzyme ACC synthase; ACC is converted to ethylene by ACC oxidase. IAA biosynthesis, both in bacteria and in plants is a complex multi-enzyme/protein process as is IAA signal transduction. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate;IAA, indole-3-acetic acid; SAM, S-adenosyl methionine. Adapted from Glick (2014)

Mechanistically, ACC deaminase producing plant growth promoting bacteria first bind to the surface of a plant (usually seeds or roots), although these bacteria may also be found on leaves and flowers or within a plant's internal tissues i.e., as an endophyte (Glick et al., 1998) Along with other small molecule components of root exudates, some of the plant ACC (a non-ribosomal amino acid) is exuded from seeds, roots or leaves (Penrose et al., 2001) and may be taken up by the bacteria associated with these tissues, and subsequently cleaved by ACC deaminase (Penrose and Glick 2003).The net result of the cleavage of exuded ACC by bacterial ACC deaminase is that the bacterium is *de facto* acting as a sink for ACC. Moreover, as a result of lowering

either the endogenous or the IAA-stimulated ACC level, the amount of ethylene that could potentially form in the plant is reduced. Subsequently, as a consequence of lowering plant ethylene levels, ACC deaminase-containing plant growth-promoting bacteria can reduce a portion of the ethylene inhibition of plant growth following a wide range of abiotic and biotic stresses. As a result, plants which grow in association with ACC deaminase-containing plant growth-promoting bacteria generally have longer roots and shoots and are more resistant to growth inhibition by a variety of ethylene-inducing stresses. As a consequence, the level of ACC within the plant is reduced and hence, the inhibitory action of ethylene is decreased. Thus, plants influenced by ACC deaminase positive PGPR are supposed to have longer roots and possibly shoot as well (Glick et al., 1997). Furthermore, the reduction of ethylene levels in plant tissues following ACC deaminase activity can cause significant morphological changes in root tissue, such as changes in root-hair length and increases in root mass, accompanied by the consequent improvement in nutrient uptake. The morphological changes in plants are greater when ACC deaminase action is coupled with the production of auxins by PGPR. The question arises, how does bacterial ACC deaminase selectively lower deleterious ethylene levels (the second ethylene peak) without affecting the small first peak of ethylene that is thought to activate plant defence responses. In this regard, ACC deaminase is generally present in bacteria at a relatively low level until it is induced, and the induction of enzyme activity is a rather slow and complex process. Immediately following an abiotic or biotic stress, the pool of ACC in the plant is low as is the level of ACC deaminase in the associated bacterium. Stress induces the induction of ACC oxidase (Fig 4) in the plant so that there is an increased flux through ACC oxidase resulting in the first (small) peak of ethylene that in turn induces the transcription of protective/defensive genes in the plant. At the same time, bacterial ACC deaminase is induced by the increasing amounts of ACC that ensue from the induction of ACC synthase in the plant so that the magnitude of the second, deleterious, ethylene peak is decreased significantly (typically by 50–90%). Because ACC oxidase has a greater affinity for ACC than does ACC deaminase, when ACC deaminase-producing bacteria are present, plant ethylene levels are dependent upon the ratio of ACC oxidase to ACC deaminase (Glick et al., 1998). That is, to effectively reduce plant ethylene levels, ACC deaminase must function before any significant amount of ACC oxidase is induced. Thus, in the absence of some other mechanism, IAA-producing bacteria

might all be expected to ultimately be inhibitory to plant growth. However, this is in fact not the case because as plant ethylene levels increase, the ethylene that is produced feedback inhibits IAA signal transduction there by limiting the extent that IAA can activate ACC synthase transcription (Morgan and Gausman 1966; Burg and Burg 1996; Pierik et al., 2006; Prayitno et al., 2006; Glick et al., 2007; Czarny et al., 2007; Stearns et al., 2012). With plant growth-promoting bacteria that both secrete IAA and synthesize ACC deaminase, plant ethylene levels do not become elevated to the same extent as when plants interact with bacteria that secrete IAA but do not synthesize ACC deaminase. In the presence of ACC deaminase, there is much less ethylene and subsequent ethylene feedback inhibition of IAA signal transduction so that the bacterial IAA can continue to both promote plant growth and increase ACC synthase transcription. However in this case, a large portion of the additional ACC that is synthesized is cleaved by the bacterial ACC deaminase. Therefore, the use of such plant growth-promoting bacteria containing ACC deaminase may prove useful in developing strategies to facilitate plant growth in stressed soil environments. And, hence, it may be possible to productively cultivate a variety of crop plants under stressed conditions without genetically manipulating plants, provided these plants are grown in the presence of a suitable PGPR. The net result of this cross-talk between IAA and ACC deaminase is that by lowering plant ethylene levels, ACC deaminase facilitates the stimulation of plant growth by IAA (Glick 2014).

2.5.1.5.1 Role of ACC deaminase in nodulation

ACC deaminase containing bacteria are relatively common in soil and have been found in a wide range of environments across the world. Indeed, the ability of bacteria to hydrolyze ACC has a competitive advantage over other soil inhabitants because it can use ACC as a N source (Jacobson et al., 1994). This hypothesis suggests that ACC may act as a unique/novel source of N for some soil bacteria. While searching for ACC deaminase positive rhizobial strains, it was found that amongst 13 different rhizobial strains, five strains displayed enzyme activity while seven strains had the *acdS* gene (Ma et al., 2003b). Conclusively, it was reported that the *Mesorhizobium* strain only expressed this activity when the bacterium was present within a root nodule. In other investigation conducted in Southern Saskatchewan, Canada, of the total 233 rhizobial strains isolated from soil samples collected from 30 different sites, nearly 12% (27 strains) displayed the ACC deaminase activity (Duan et al., 2009). Similarly, ACC deaminase genes have been reported in chickpea *Mesorhizobium*

isolates (Nascimento et al., 2012a), *B. japonicum* E109, USDA110 and SEMIA5080 (Boiero et al., 2007). Rhizobial strains that express ACC deaminase are up to 40% more efficient at forming nitrogen-fixing nodules than strains that lack this activity (Ma et al., 2003a, 2004). However, strains of rhizobia that express ACC deaminase have only a low level of enzyme activity compared with free-living plant growth-promoting bacteria, i.e. typically around 2–10%. Thus, free-living bacteria bind relatively non-specifically to plant tissues (mainly roots) and have a high level of ACC deaminase activity that can protect plants from different abiotic and biotic stresses by lowering ethylene levels throughout the plant. On the other hand, (symbiotic) rhizobia that generally bind tightly only to the roots of specific plants, have a low level of enzyme activity which facilitates nodulation by locally lowering ethylene levels. It is not known whether the large differences in enzyme activity that are observed when comparing free-living bacteria with rhizobia is a consequence of differences in the amount of enzyme synthesized by one type of bacteria versus the other or of differences in the specific catalytic activity of the enzymes from the different types of bacteria. It has also been observed that some rhizobia reduces the plant ethylene levels mediated by ACC deaminase activity and enhances nodulation in host legumes (Zahir et al., 2008; Belimov et al., 2009) or modifies root system of non-legumes. For instance, strains of *R. leguminosarum* bv. *viciae* and *M. loti* increased the number of lateral roots in *Arabidopsis thaliana* because of this plant growth-promoting mechanism (Contesto et al., 2008). In addition to the more common mode of *acdS* transcriptional regulation, *acdS* genes from various strains of *Mesorhizobium loti* have been found to be under the transcriptional control of the *nifA* promoter that is normally responsible for activating the transcription of nif, nitrogen fixation genes (Kanekoet al., 2000; Sullivanet al., Uchiumi et al., 2004; 2002; Nukui et al., 2006; Nascimento et al., 2012a). The consequence of this somewhat unusual mode of regulation is that, unlike ACC deaminases from other rhizobia, the *M. loti* ACC deaminase does not facilitate nodulation but, rather, is expressed within nodules. The result of this unusual regulation is, in *M. loti*, ACC deaminase may act to decrease the rate of nodule senescence. This is particularly important because of the fact that nitrogen fixation, a process that utilizes a very high level of energy in the form of ATP, could (perhaps inadvertently) activate stress ethylene synthesis resulting in premature nodule senescence.

2.5.1.6 Microbial phosphate solubilization: Current perspectives

Of the major plant nutrients, phosphorus (P) is required for various metabolic processes such as, energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration (Fernandez et al., 2007; Zaidi et al., 2009; Elser 2012). After uptake by plants, P also stimulates root development and facilitates flower formation and quality and quantity of fruits and seed formation (Ahemad et al., 2009). Phosphorus however, is also one of the major nutrients limiting plant growth (Fernandez et al., 2007). Worldwide, 5.7 billion hectares land contain too little available P for sustaining optimal crop production (Hinsinger 2001; Khan et al., 2013) P concentration in most soils ranges from 0.1 to 10 µM; P required for optimal growth ranges from 1 to 5 µM for grasses and 5 to 60 µM for high demanding crops such as tomato and pea (Raghothama 1999). Sub-optimal levels of P, can however, lead to a 5 to 15% losses in the yield of crops (Hinsinger 2001). Phosphorus is present in the soils both in organic and inorganic forms, of which organic forms is found in humus and other organic materials including decayed plant, animal and microbial tissues. Organic P is an important reservoir of immobilized P accounting for about 20–80% of total soil P (Richardson et al., 2009). Phosphorus in labile organic compounds can be slowly mineralized as available inorganic P or it can be immobilized as part of the soil organic matter (Mckenzie and Roberts 1990). The process of mineralization or immobilization is carried out by microorganisms and is highly influenced by soil moisture and temperature. Mineralization and immobilization are most rapid in warm, well-drained soils (Busman et al., 2002). Soil inorganic P is however, controlled mainly by solution pH and the concentration of cations and in most soils, maximum P availability occurs between pH 5.5 to 7. Within this pH range, P is fixed by hydrous oxides of Fe, Al, and Mn while between pH 6 to 8 and pH 6.5 to 8.5, P is fixed by silicate minerals and Ca, respectively. As a consequence, the most efficient use of P in reutral and calcareous soils occurs between pH 6 to 7. However, the majority of P is unavailable for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils. Therefore, phosphatic fertilizers are applied to soil to replenish the P demands of growing plants. However, a large portion of soluble inorganic P applied to the soil as fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein 1986). For instance, in the United States, an average 29% of P added in fertilizer and manure is removed by harvesting crops (Sharpley 2006). Moreover, the concentration of soluble P in soil solution is very low (400–

1,200 mg kg^{-1} of soil) (Ehrlich 1990; Rodriguez and Fraga 1999). Attempts to overcome the P deficiency by applying phosphatic fertilizers is expensive and ecologically unsafe practice because the efficiency of the added P fertilizer is as low as about 10% (Werft and Dekkers 1996). Therefore, order to reduce chemical addition to soils and spiralling cost, and undeniable deleterious environmental impacts of P fertilizers, there is an urgent need to find a suitable/feasible alternative to chemical fertilizers. This has led to search for environment-friendly and economically feasible alternative strategies for improving crop production in low P soils. In this context, organisms endowed with phosphate solubilizing activity, often termed phosphate solubilizing microorganisms (PSM), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers (Ahemad and Khan 2010; Xiang et al., 2011; Hui et al., 2011; Khan et al., 2013). Of the various PSM (s) inhabiting rhizosphere, phosphate-solubilizing bacteria (PSB) are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available (Khan et al., 2006c). Though, PSB are commonly found in most soils (Wani et al., 2007c; Yi et al., 2008; Behbahani 2010; Ahemad and Khan 2011b; Marra et al., 2011; Sanjotha et al., 2011; Yadav et al., 2011) their establishment and performances are severely affected by environmental factors (Tilak 1991; Wani et al., 2007c). However, the beneficial effects of the inoculation with PSB, used either alone (Chen et al., 2008, Singh et al., 2008; Poonguzhali et al., 2008) or in combination with other rhizospheric microbes have been reported (Zaidi et al., 2003; Zaidi and Khan 2005; Zaidi and Khan 2006; Vikram and Hamzehzarghani 2008).

2.5.1.6.1 Mechanism of P- solubilisation: A brief account

The insoluble forms of P such as tri-calcium phosphate $(Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4) etc. may be converted to soluble P by P-solubilizing bacteria inhabiting different soil ecosystems (Song et al., 2008; Ahemad and Khan 2011b; Khan et al., 2013). Soil microorganisms in this regard have generally been found more effective in making P available to plants from both inorganic and organic sources by solubilizing (Toro 2007; Wani et al., 2007b) and mineralizing difficultly available P (Ponmurugan and Gopi 2006; Bishop et al., 1994;), respectively. Several workers have documented their findings in order to better understand as to how the microbial populations including bacteria cause the solubilization of insoluble P (Cunningham and Kuiack 1992; Illmer and Schinner

1995; Buch et al., 2008; Song et al., 2008). Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids (OA) secreted by microorganisms have been well recognized and widely accepted theory as a principal means of P solubilisation (Maliha et al., 2004). The OA produced by bacterial cultures (Table 5) in the natural environment or under *in vitro* conditions chelate mineral ions or decrease the pH to bring P into solution (Maliha et al., 2004; Pradhan and Sukla 2005). Consequently, the acidification of microbial cells and their surrounding leads to the release of P-ions from the P-mineral by H^+ substitution for Ca^{2+} (Goldstein 1994). However, there are also reports which suggest that insoluble P could be transformed into soluble forms of P without OA production by microbes (Asea et al., 1988; Illmer and Schinner 1992, 1995; Chen et al., 2006). For example, Altomare et al., (1999) while investigating the P solubilizing ability of plant growth-promoting and bio-control fungus *Trichoderma harzianum* T-22 did not record OA production (rock P was used as insoluble P source) under *in vitro* condition. It was concluded from this study that the insoluble P could be solubilized by mechanisms other than acidification process (Fig. 5) also; the fungal-solubilizing activity was credited both to chelation and to reduction processes, which may be useful in the management of phytopathogens. Apart from the OA theory, some of the inorganic acids (Reyes et al. 2001; Richardson 2001) such as HCl (Kim et al., 1997), nitric acid and sulfuric acids (Dugan and Lundgren 1965) produced by chemoautrophs and the H^+ pump for example in *Penicillium rugulosum*, have also been reported to solubilize the insoluble P (Reyes et al., 1999). The inorganic acids convert tri-calcium phosphate to di and monobasic phosphates with the net result of an enhanced availability of the element to plants.

Table 5- Organic acid production and P solubilization by PS bacteria

PS bacteria	Organic acid produced	Initial pH	Final pH	Amount of P slubilized (µg/ml)	Time (h)	Reference
Pseudomonas trivialis (BIHB 769)	GA, 2-KGA, LA, SA, FA, MA	7±0.2	3.70	806.4±2.3	120	Vyas and Gulati (2009)
P. poae (BIHB 808)	GA, 2-KGA, SA, CA, MA	7±0.2	3.58	821.4±1.7	120	Vyas and Gulati (2009)
P. flourescens (BIHB 740)	GA, 2-KGA, SA, FA, CA, MA	7±0.2	3.97	768.3±2.6	120	Vyas and Gulati (2009)
Pseudomonas spp. (BIHB 751)	OA, GA, 2-KGA, FA, MA	7±0.2	4.20	318.7±2.0	120	Vyas and Gulati (2009)
Enterobacter Hy-401	OA, GA, MA, LA, CA, SA, FuA	7-7.5	4.32±0.02	623.6±23.0	120	Yi et al., (2008)
Arthrobacter Hy 505	OA, GA, LA, CA	7-7.5	5.50±0.04	428.9±15.3	120	Yi et al., (2008)
Azotobacter Hy - 510	OA, GA, TA, LA, SA, FuA	7-7.5	4.69±0.05	229.03±15.2	120	Yi et al., (2008)
Enterobacter Hy-402	OA, GA, TA, CA, SA, FuA	7-7.5	4.51±0.02	111.73±8.07	120	Yi et al., (2008)
Rhodococcus erythropolis (CC-BC11)	GA	7-6.8	5.3	186.9	72	Chen et al., (2006)
Bacillus megaterium (CC-BC10)	CA, LA, PA	7-6.8	5.1	270.2	72	Chen et al., (2006)
Arthrobacter sp. (CC-BC03)	CA, LA	7-6.8	4.9	519.7	72	Chen et al., (2006)
A. ureafaciens (CC-BC02)	CA	7-6.8	5.0	316.1	72	Chen et al., (2006)
Serratia marcescens (CC-BC14)	CA, GA, SA, LA	7-6.8	4.9	421.8	72	Chen et al., (2006)
Delftia (CC-BC21)	SA,	7-6.8	4.9	346.1	72	Chen et al., (2006)
Chryseobacterium (CC-BC05)	CA	7-6.8	6.0	298.9.	72	Chen et al., (2006)
Phyllobacterium myrsinacearum (CC-BC19)	GA	7-6.8	5.2	201.2	72	Chen et al., (2006)
Gordonia (CC-BC07)		7-6.8	6.0	31.5	72	Chen et al., (2006)
Enterobacter intermedium	2-KGA	8	2.8	65×10^3	240	Hwangbo et al., (2003)
Bacillus amyloliquefaciens	AA, IBA, IVA, LA, SA	ND	ND	60 (approx)	24	Vazquez et al., (2000)
B. atrophaeus	PA, IBA, IVA, VA, ISA, SA	ND	ND	110 (approx)	24	Vazquez et al., (2000)
B. licheniformis	IBA, VA, LA, FuA, SA	ND	ND	105 (approx)	24	Vazquez et al. ,(2000)
V. proteolyticus	AA, LA	ND	ND	475 (approx)	24	Vazquez et al., (2000)
P. macerans	IBA, IVA, LA, SA	ND	ND	85 (approx)	24	Vazquez et al., (2000)
X. agilis	IBA, IVA, LA	ND	ND	190 (approx)	24	Vazquez et al., (2000)

Gluconic acid- GA, 2-KGA-2α-ketogluconic acid, Lactic acid- LA, Succinic acid- SA, Formic acid-FA, Malic acid- MA, Citric acid- CA, Oxalic acid-OA, Fumaric acid-FuA, Tartaric acid-TA, Propionic acid-PA, Acetic acid-AA, Isobutyric acid-IBA, Isovaleric acid-IVA, Valeric acid-VA, Isocaproic acid-ISA, ND not determined (Adapted from Khan et al., 2013).

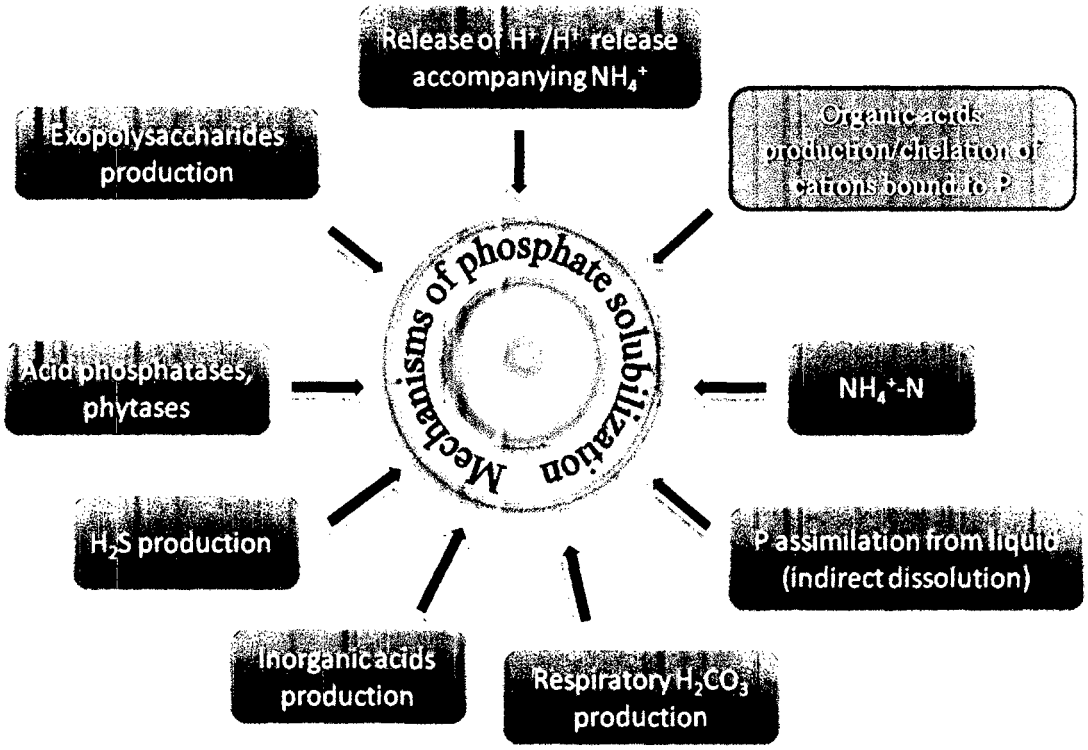

Fig. 5 Mechanisms of P solubilization by phosphate solubilizing bacteria (Adapted from Ahemad 2009)

2.5.1.6.2 Functional diversity among phosphate solubilizing bacteria

Phosphate solubilizing microorganisms increases the overall performance of plants by providing mainly soluble P to plants in different production systems. However, they also benefit plants by other mechanisms (Fig 6). Indeed, PSM exhibit multifunctional properties (Vikram et al., 2007; Vassileva et al., 2010; Singh et al., 2010; Ahemad and Khan 2010; Yadav et al., 2011; Khan et al., 2012) for example they are known to synthesize siderophores (Matthijs et al., 2007; Hamdali et al., 2008; Viruel et al., 2011) IAA and gibberellic acid (Sattar and Gaur 1987; Souchie et al., 2007; Viruel et al., 2011). Phosphate solubilizing bacteria such as Gram-negative *P. fluorescens, P. aeruginosa,* and *Chromobacterium violaceum* also secretes cyanide, a secondary metabolite which is ecologically important (Wani et al., 2007a) and gives a selective advantage to the producing strains (Rudrappa et al., 2008). Besides strict P solubilizers, a few genera of rhizobia for example *Bradyrhizobium* and *Rhizobium* have also been found to solubilize P and secrete IAA (Pandey et al., 2007; Badawi et al., 2011). Interestingly, the ability of PSB for example *Serratia marcescens* to secrete siderophores and cyanide are critical in managing various diseases inflicted by the plant pathogens (Vassilev et al., 2006) and indirectly promoting the plant growth

(Badawi et al., 2011). Some of the compounds synthesized by P-solubilizing bacteria with possible effect on plant growth promotion are listed in Table 3 and 4.

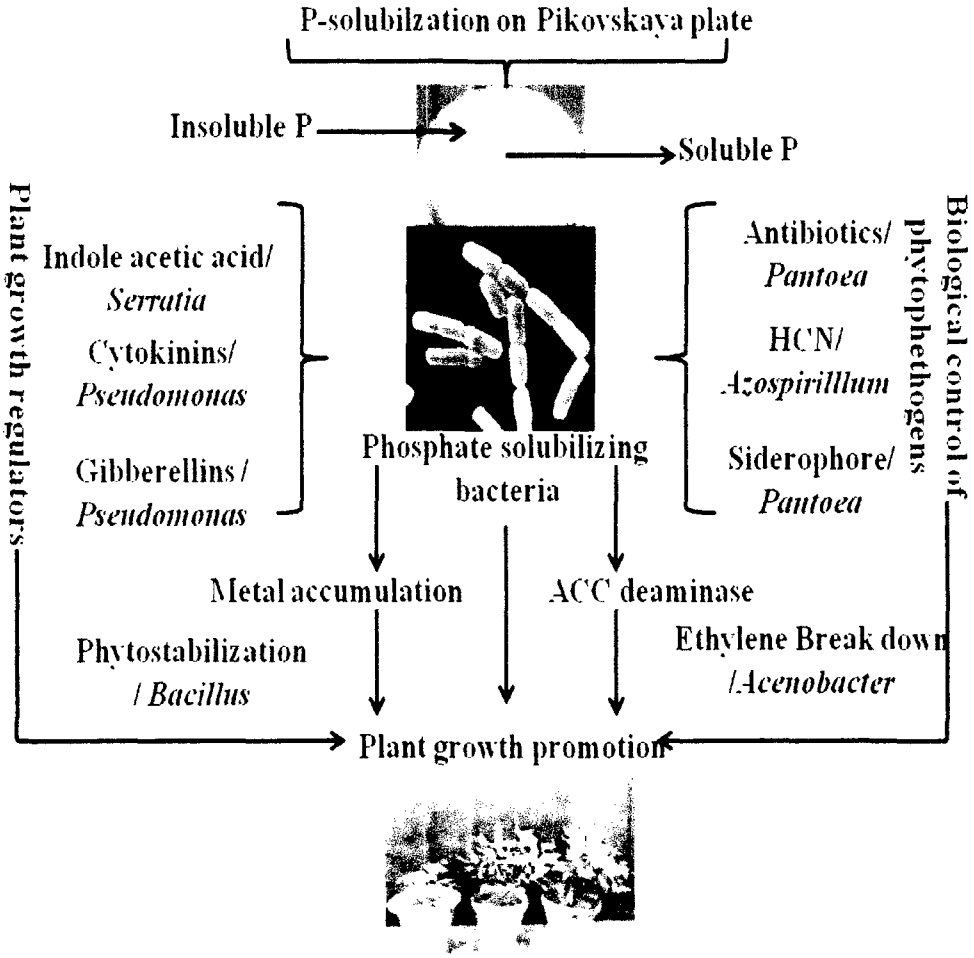

Fig. 6 · An illustration depicting functional diversity among PS bacteria (Modified from Oves et al., 2009; photograph of PSB, courtesy M. Oves)

2.5.1.7 Siderophores: A general outlook

Iron is essential for almost all life for processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil is limited by the very low solubility of the Fe^{3+} ion. It accumulates in common mineral phases such as iron oxides and hydroxides and hence cannot be readily utilized by organisms. Microbes (e.g., bacteria and fungi) release siderophores (Greek: "iron carrier"), a small (generally less than 1000 molecular weight) high-affinity iron chelating compounds, to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. There are more than 500 different siderophores which are produced mainly by Gram-positive and Gram-

negative bacteria. Siderophores are highly electronegative and bind Fe (III), preferentially forming a hexacoordinated complex. The iron ligation groups have been tentatively classified into three main chemical types: hydroxamate (e.g., aerobactin and ferrichrome), catecholates/phenolates (e.g., enterobactin) and hydroxyl acids/ carboxylates (e.g., pyochelin). Some siderophores contain more than one of these three iron-chelating groups (Table 6). Siderophores are however, usually classified by the ligands used to chelate the ferric iron. Citric acid can also act as a siderophore. The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores (Fig. 8) which cannot be transported by other microbes specific active transport systems, or in the case of pathogens deactivated by the host organism. Siderophores are important for some pathogenic bacteria for their acquisition of iron. The strict homeostasis of iron leads to a free concentration of about 10^{-24} mol l^{-} and hence there are great evolutionary pressures put on pathogenic bacteria to obtain this metal. For example, the anthrax pathogen *Bacillus anthracis* releases two siderophores, bacillibactin and petrobactin, to scavenge ferric iron from iron proteins. The effects of microbial siderophores on growth and development of plants are presented in Fig. 7

Table 6- Some examples of siderophores produced by various bacteria and fungi

S.No.	Siderophores	Organisms
1	**Hydroxamate**	
A	Ferrichrome	*Ustilago sphaerogena*
B	Desferrioxamine B	*Streptomyces pilosus*
	(Deferoxamine)	*Streptomyces coelicolor*
C	Desferrioxamine E	*Streptomyces coelicolor*
D	fusarinine C	*Fusarium roseum*
E	Ornibactin	*Burkholderia cepacia*
2	**Catecholate**	
A	Enterobactin	*Escherichia coli*
B	Bacillibactin	*Bacillus subtilis*
		Bacillus anthracis
C	Vibriobactin	*Vibrio cholera*

3	**Mixed ligands**	
A	Azotobactin	*Azotobacter vinelandii*
B	Pyoverdine	*Pseudomonas aeruginosa*
C	Yersiniabactin	*Yersinia pestis*

2.5.1.7.1 Siderophores and BNF

Siderophore produced by majority of PGPR (Rajkumar et al., 2010) including rhizobia (Ahemad and Khan 2012) has been suggested as one of the modes of growth promotion of nodulated legumes under field conditions wherein siderophores facilitate the uptake of iron (assimilation) from the environment (Katiyar and Goel, 2004a, Kloepper and Schroth 1978). The iron enzymes involved include nitrogenase, leghemoglobin, ferredoxin and hydrogenase with nitrogenase and leghemoglobin constituting up to 12% and 30% of total protein in the bacterial and infected plant cells, respectively (Verma and Long 1983). A nodulated legume has been found to have an increased demand for iron compared to that of a non-nodulated plant (Deryto and Skorupska 1993). For example, *Pseudomonas* sp. strain 267 enhanced symbiotic N_2 fixation in clover under gnotobitoic conditions, produced fluorescent siderophores under low-iron conditions and secreted B group vitamins (Marek-Kozaczuk and Skorupska 2001). However, Tn5 insertion mutants of strain 267 defective in siderophore production did not differ from the wild-type in promoting the growth of clover suggesting that the siderophore production had no effect on stimulating nodulation. In contrast, Gill et al., (1991) demonstrated that mutants of *R. melioti* that were unable to produce siderophores were able to nodulate the plants, but the efficiency of N_2 fixation was less compared to the wild-type, indicating the importance of iron in N_2 fixation. In a similar study, *Kluyvera ascorbata*, a siderophore-producing PGPR, was able to protect plants from heavy metal toxicity (Burd et al., 1998).

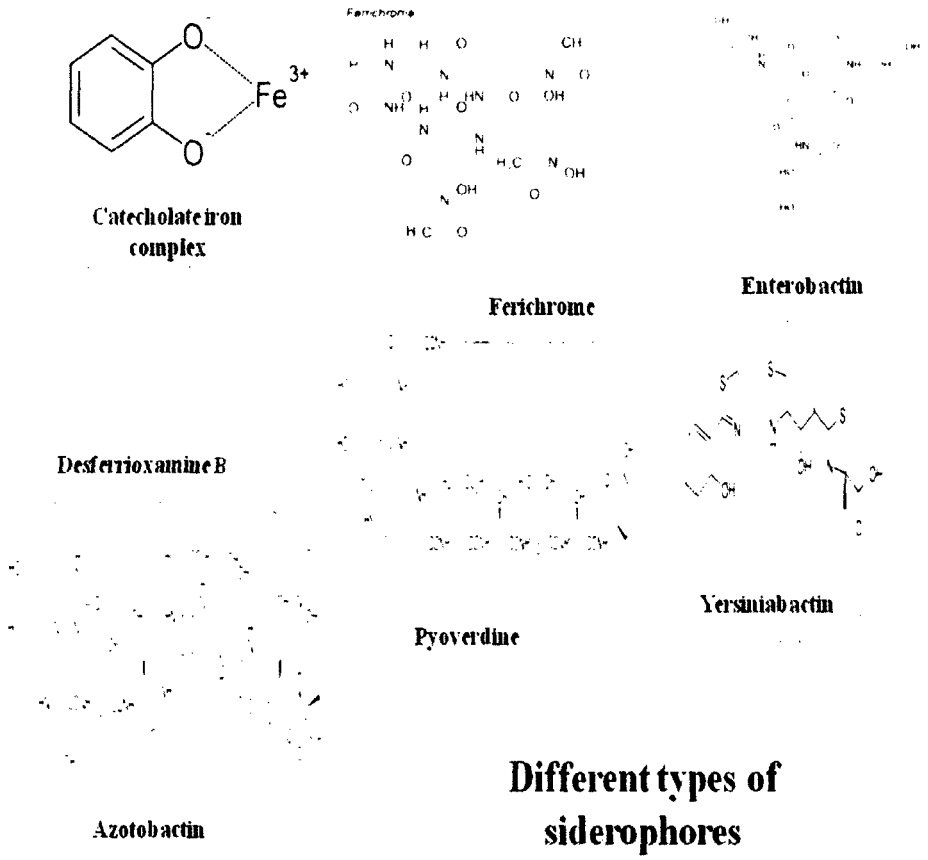

Fig. 7 : Different types of siderophores

2.5.1.8 Antibiotics production by plant growth promoting rhizobacteria

Plant growth promoting rhizobacteria also promotes the growth of plants by secreting antimicrobial compounds, induction of systemic resistance (ISR), and production of pathogen-related (PR) proteins (Compant et al., 2005). Antibiotic production by biocontrol PGPR is perhaps the most powerful mechanism against phytopathogens (Bashan and de-Bashan 2005), and the first clear-cut experiment demonstrating the role of PGPR in suppression of plant disease through antibiotic production was reported by Tomashow and Weller (1988). These antibiotics may be antitumor, antiviral, antimicrobial, antihelmenthic, and cytotoxic (Fernando et al., 2005). The antibiotics can also contribute to microbial competitiveness besides their role in suppressing the growth of plant root pathogens. The PGPR strains that produce these compounds are, therefore, of considerable interest as biological control agents (Thomshow et al., 2003) and provide an alternative to chemical pesticides. Several antimicrobial compounds belonging to polypeptides, heterocyclic nitrogenous compounds, and lipopeptides groups active against phytopathogens have been

reported (Thomshaw and Webler 1995). In addition, plants can acquire local and systemic resistance to diseases through various biological agents, including necrotizing pathogens, nonpathogens, and soil-borne rhizosphere bacteria and fungi (Van Loon et al., 1998). This type of resistance, known as induced systemic resistance, is mediated by a jasmonate/ethylene sensitive pathway (Van Loon et al., 1998). Induction of systemic resistance has been established as a new mechanism by which plants defend themselves against pathogen attachment. Various reports confirm the induction of systemic resistance by PGPR. For instance, PGPR strains, i.e., *P. putid*a (strain 89B-27), *S. marcescens* (strain 90-166), *Flavomonas oryzihabitans* strain (INR-5), and *Bacillus pumilus* (strain INR-7), have significantly reduced populations of the striped cucumber (Zehnder et al., 1997). Furthermore, the combined inoculation of PGPR (*Bacillus* and *Pseudomonas*) and *Rhizobium* sp. increased the production of defense-related enzymes, i.e., L-phenylalanine ammonia lyase (PAL), POX and polyphenol oxidase (PPO), in co-inoculated pigeonpea plants which in turn decreased the dry weight of mycelium and fusaric acid production by fusarial wilt of pigeonpea, suggesting that the PGPR and rhizobia could be used together for induction of systemic resistance against fusarial wilt in pigeon pea (Dutta et al., 2008). Antibiosis and antagonistic activities of PGPR recovered from wheat and rice seeds, corn plants, and potato have been suggested as possible mechanisms of growth inhibition of various phytopathogens (Lodewyckx et al., 2002; Rosenblueth and Martı´nez-Romero 2006). For instance, *P. fluorescens* capable of synthesizing 2,4-diacetyl phloroglucinol (DAPG) has shown the production of antimicrobial compounds in planta conditions. Similarly, production of antibiotics phenazine, pyocyanine and DAPG by *Pseudomonas* spp. associated with induced systemic resistance (ISR) activity in sugarcane against red rot disease has been reported (Viswanathan and Samiyappan 2004). However, the bacterial strains varied in their capability to produce the metabolites. The purified compounds tested for their antifungal activity completely arrested the conidial germination and mycelia growth of red rot pathogen, *Colletotrichum falcatum*, suggesting that the metabolites played an important role in antagonism/ISR. Similarly, *Bacillus lentimorbus* and *Bacillus cereus* isolated from coffee plant demonstrated inhibitory effects against coffee rust pathogen *Hemileia vastatrix* and significantly enhanced the coffee production. The pathogen suppression was suggested to be due possibly to the synthesis of a significant amount of fungal cell wall lysing enzymes, antibiosis, competition, and

ISR in host (Shiomi et al., 2006). Recently, PGPR bioformulations of *Pseudomonas* and *Bacillus* were tested for their efficacy against blister blight (*Exobasidium vexans*) disease in tea under field conditions for two seasons. Among the bioformulations tested, foliar application of *Pseudomonas fluorescens* Pf1 at 7-day intervals consistently reduced the disease incidence of blister blight for two seasons, almost comparable with that of chemical fungicide. In addition to disease control, it also increased tea yield significantly compared to the untreated control. Defense enzymes, such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase, b-1,3-glucanase, and phenolics were found more in P. fluorescens Pf1- treated plants compared to control. This finding revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations (Saravanakumar et al., 2007).

2.5.1.9 Production of cyanogenic compounds

Cyanide is yet another secondary metabolite produced during the early stationary growth phase (Knowles and Bunch 1986) by several PGPR, notably *Pseudomonas* spp. and *Bacillus* (Wani et al., 2007c; Ahmad et al., 2008), *Chromobacterium* (Faramarzi and Brand 2006), and *Rhizobium* spp. (Wani et al., 2008a, 2008b) by oxidative decarboxylation pathway using glycine, glutamate, or methionine as precursors (Curl and Truelove 1985). The cyanide so released by microbial communities in solution acts as a secondary metabolite and confers a selective advantage on the producer strains (Vining 1990). Although cyanide is a phytotoxic agent capable of disrupting enzyme activity involved in major metabolic processes, its role as a biocontrol substance is overwhelming (Voisard et al., 1989; Devi et al., 2007). Hydrogen cyanide (HCN) among cyanogenic compounds effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly pseudomonads, are reported to be resistant (Bashan and de-Bashan 2005).

2.5.1.10 Production of lytic enzymes

A variety of other microbial compounds are involved in the suppression of phytopathogenic growth leading therby to the reduction in damage to plants. These microbially synthesized compounds include defence enzymes, such as, chitinase, ß-1,3-glucanase, peroxidise, protease and lipase (Karthikeyan et al., 2006; Bashan and

de-Bashan 2005). Chitinase and β-1,3-glucanase degrade the fungal cell wall and cause lysis of fungal cell. Furthermore, chitin and glucan oligomers released during degradation of the fungal cell wall by the action of lytic enzymes act as elicitors that elicit various defense mechanisms in plants (Karthikeyan et al., 2005). Such enzymes produced by *Pseudomonas stutzeri* have demonstrated the lysis of the pathogen *Fusarium* sp. (Bashan and de- Bashan 2005). Peroxidase represents another component of an early response in plants to pathogen attack and plays a key role in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and West 1989). In bean, rhizosphere colonized by various bacteria induced PO activity (Zdor and Anderson 1992). In a study, a rapid increase in PO activity was recorded in coconut (*Cocos nucifera* L.) treated with a mixture of *P. fluorescens*, *T. viride* and chitin which contributed to induced resistance against invasion by *Ganoderma lucidum*, the causal agent of *Ganoderma* disease (Karthikeyan et al., 2006). These findings suggest that PGPR possessing the ability to synthesize hydrolytic enzymes can effectively be utilized for managing the plant diseases and can help to reduce the pesticide usage.

2.6 PGPR-Crop interactions: Importance in sustainable agriculture

The intrinsic yet diverse functional ability of PGPR for enhancing growth and yields of various crops including legumes has been explored extensively and well documented in literature (Table 7). Even-though the results obtained under both pot and field trials have been conflicting, these organisms are being promoted largely as an alternative to chemical synthetic fertilizers. Sadly, the majority of such studies have been conducted under controlled environment than under natural field conditions. Considering the potential of functionally variable yet agronomically effective PGPR it has become important to find some new strain possessing multiple plant growth promoting activities so they could be transferred to ultimate users for enhancing the production of crops in different agro-ecological niches. In this context, numerous PGPR have been isolated and when tested have been found to exhibit larger impact when they were put back/re-applied into the same habitat/soil where from they were isolated. Therefore, there is need to develop area/location specific preparation of bacterial fertilizers often termed biofertilizers so that such microbes faces little competition from natural inhabitants of the area and climatic/seasonal variation (Chanway and Holl 1992; Nowak 1998; Ahemad and Khan 2010b). When such practice is adopted, there is likelihood that such microbes (introduced PGPR) will

adapt to the rhizosphere more easily and rapidly. However, the failure in some crops to respond properly and effectively have been reported due to the use of poor quality and reduced shelf life of PGPR, lack of standard delivery systems and/or failure in maintaining a required density of PGPR onto seeds or roots. Apart from these biofertilizers related factors, the nature and composition of the material used as a carrier for inoculants preparation along with fluctuating environments and injudicious application of fertilizers in agriculture practices may also result in significant adverse impact on the crop production under different agro-ecosystems (Shaharoona et al., 2008; Ahemad and Khan 2010b). And hence, a deeper understanding of as to how PGPR functions in different situations under diverse climatic conditions and against which crop they show greater results (plant specificity) is likely to provide option to design and develop some better quality PGPR inoculants. Therefore, considering all these, there is urgent need to focus on to the management of plant-microbe interactions, especially in regard to their variable functional properties and greater adaptability to conditions under changing environments for the benefit of plants. Furthermore, scientists need to address certain issues like, how to improve the efficacy of biofertilizers, what should be an ideal and universal delivery systems, how to stabilize these microbes in soil systems, and how nutritional and root exudation aspects could be controlled in order to get maximum benefits from PGPR application.

Table 7: Plant responses to inoculation with PGPR

PGPR	Plants	Comments	References
Azospirillum brasilense Az1 and Az2, *P. fluorescens* Pf	*Oryza sativa* L	The inoculation increased aerial and root biomass and grain yield by 12, 40 and 16% respectively over uninoculated control.	Naiman et al., (2009)
A. brasilense, *Pantoea dispersa*	*Capsicum annuum*	Inoculation increased the concentration of citric, ascorbic and succinic acids in green fruit of sweet pepper compared with non-inoculated control.	Amor et al., (2008)
Bacillus sp.	*Solanum tuberosum*	Bacterial inoculation caused increment in the growth of the plants as compared to the non-inoculated treatments.	Ahmed and Hasnain (2008)
P. fluorescens, *B. subtilis*, *Sinorhizobium meliloti*, *Bradyrhizobium* sp.	*Origanum majorana* L.	Only *P. fluorescens* and *Bradyrhizobium* sp. showed significant increases in shoot length, shoot weight, number of leaves and node, and root dry weight, in comparison to control plants or plants treated with other PGPR. Essential oil yield was also significantly increased relative to non-inoculated plants, without alteration of oil composition.	Banchio et al., (2008)
Phyllobacterium brassicacearum STM196, *P. putida* UW4, *R. leguminosarum* bv. viciae 128C53K, *Mesorhizobium loti*	*Arabidopsis thaliana*	Root hairs of seedlings inoculated with the ACC-deaminase strains were significantly longer.	Contesto et al., (2008)
Rhizobium tropici (CIAT899), *Paenibacillus polymyxa* (DSM 36), *Rhizobium*, *P. polymyxa* strain Loutit (L), *Paenibacillus*, *Bacillus* sp.	*Phaseolus vulgaris* L.	Beans coinoculated with *R. tropici* (CIAT899) and *P. polymyxa* (DSM 36) had higher leghemoglobin concentrations, nitrogenase activity and N_2 fixation efficiency and thereby formed associations of greater symbiotic efficiency. Inoculation with *Rhizobium* and *P. polymyxa* strain Loutit (L) stimulated nodulation. PGPR also stimulated specific-nodulation (number of nodules per gram of root dry weight) and increased accumulated N.	Figueiredo et al., (2008)
Bacillus strains	*Capsicum annuum*	Stem diameter, root elongation, root dry weight, shoot dry weight and yield were increased in response to inoculation in the field experiment by 7.0%-20.5%, 7.0%-17.0%, 4.5%-23.5%, 16.5%-38.5%, and by 11.0%-33.0%, respectively.	Mirik et al., (2008).,
Pseudomonas spp.	*Triticum aestivum* L.	Inoculation significantly increased growth, yield and nutrient use efficiency of wheat.	Shaharoona et al., (2008)
Serratia oderifera (J118), *Pantoea dispersa* (J112), *Enterobacter gergoviae*	*Cicer arietinum* L.	The PGPR in the presence of P-enriched compost resulted in a highly significant increase in fresh biomass (84%), number of pods plant^{-1} (97%), grain yield (79%) and number of nodules plant^{-1} (87%)	Shahzad et al., (2008)

PGPR	Plants	Comments	References
(J107)		compared to uninoculated control.	
PGPR strains OSU-142, OSU-7, BA-8 and M-3)	*Malus domestica* Borkh)	Inoculation with OSU-142, OSU-7, BA-8 and M-3 PGPR increased average shoot length by 59.2, 18.3, 7.0 and 14.3% relative to the control and fruit yield by 116.4, 88.2, 137.5 and 73.7%, respectively. Bacterial inoculation increased shoot diameter from 7.0 to 16.3% compared to control.	Aslantas et al., (2007)
P. fluorescens, *P. fluorescens* subgroup G strain 2, *P. marginalis*, *P. putida* subgroup B strain 1 and *P. syringae* strain 1)	*Lycopersicon esculentum*	*Pseudomonas putida* was shown to improve fruit yields in rockwool and in organic medium. The production of IAA was shown as possible mechanism for plant growth stimulation by the bacterium. In addition, roots of tomato seedlings grown in the presence of increasing concentrations of IAA were significantly longer when seeds were treated with *P. Putida*.	Gravel et al., (2007)
B. megaterium, *B. subtilis*, *Pseudomonas corrugate*	*Zea mays* L.	All the three bacterial inoculants resulted in an increment in grain yield of maize up to 122%, 135% and 194%, respectively, as compared to respective control. The overall beneficial effects of bacterial inoculations were contributed to the colonization and survival of the introduced bacteria, and stimulation of the indigenous microflora in the rhizosphere.	Kumar et al., (2007)
B. subtilis BEB-ISbs (BS13)	*Lycopersicon esculentum*	Yield per plant, fruit weight and length were increased significantly by the *Bacillus subtilis* BEB-ISbs (BS13) treatment when compared to the control.	Mena-Violante and Olalde-Portugal (2007)
Pseudomonas spp., *Burkholderia caryophylli*	*Triticum aestivum* L.	Both PGPR containing ACC-deaminase positively influenced on growth and yield of wheat.	Shaharoona et al., (2007b)
B. pumilus 8N-4		Inoculation of the wheat variety Orkhon with PGPR *B. pumilus* 8N-4 (originated from Mongolia) resulted in the maximum increase in plant biomass, root length, and total N and P contents in plants. The isolate was also capable of producing auxin and siderophore.	Hafeez et al., (2006)
Cyanobacterial strains	*Oryza sativa* L.	Significant increases in grain and straw yield were observed when rice seedlings were inoculated with four cyanobacterial strains either applied alone or in combination with chemical fertilizer. In addition, a saving of 25 kg N ha^{-1} was attained through cyanobacterial fertilization.	Jha and Prasad (2006)
Pseudomonas spp.	*Zea mays* L., *Vigna radiata*	Significant increases in plant height, root weight and total biomass were observed in response to inoculation with PGPR containing ACC-	Shaharoona et al., (2006a, b)

PGPR	Plants	Comments	References
		deaminase. Similarly, inoculation significantly improved grain yield of maize in the presence of nitrogenous fertilizers. Effect of PGPR was also positive on nodulation of mung bean (*Vigna radiata*).	
Pseudomonas, *Azotobacter*, and *Azospirillum*	*Triticum aestivum* L.	Significant positive effects of inoculation on germination and growth of wheat were observed.	Shaukat et al., (2006)
A. chroococcum, *B. megaterium*, *B. mucilaginous*	*Zea mays* L.	The application of PGPR significantly increased the plant growth and resulted in the highest biomass and seedling height. Inoculation not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil.	Wu et al., (2005)
Pseudomonas spp.	*Arachis hypogaea* L.	Seed inoculation with PGPR containing ACC-deaminase resulted in a significantly higher pod yield than the control, in pots, during rainy and post-rainy seasons. PGPR also significantly enhanced pod yield (23–26%, 24–28% and 18–24%, respectively), haulm yield and nodule dry weight over the control under field conditions.	Dey et al., (2004)
B. licheniformis CECT 5106, *B. pumilus* CECT 5105	*Quercus ilex* ssp. Ballota	Only *B. licheniformis* promoted the growth of *Q. ilex* seedlings. Furthermore, *B. licheniformis* inhibited fungal growth as revealed by ergosterol/chitin analysis.	Domenech et al., (2004)
PGPR isolates	*Triticum aestivum* L.	Peat based seed inoculation with selected PGPR strains capable of producing auxins exhibited stimulatory effects on grain yields of tested wheat cv. in pot (up to 14.7% increase over control) and field experiments (up to 27.5% increase over control); however, the response varied with cv. and PGPR strains. It was concluded that the strain, which produced the highest amount of auxins in nonsterilized rhizosphere soil, also caused maximum increase in growth and yield of both the wheat cultivar.	Khalid et al., (2004a)
PGPR	*Quercus ilex* spp. Ballota, *Pinus pinea*	All strains significantly increased stem length, neck diameter and shoot dry weight of the inoculated plants.	Garci'a et al., (2003)
Enterobacter cloacae, *P. putida*, *P. fluorescens*	*Brassica rapa*	Inoculation significantly enhanced root elongation of canola under gnotobiotic conditions.	Penrose and Glick (2003)
Rhizobacteria	*Brassica juncea*	A significant increase in growth was observed in the inoculated seedlings. The PGPR were also capable of producing IAA.	Asghar et al., (2002)

PGPR	Plants	Comments	References
P. putida Am2, *P. putida* Bm3, *Alcaligenes xylosoxidans* cm4, *Pseudomonas* sp. Dp2	*Brassica juncea* L.	Significant increase in root elongation of phosphorus-sufficient seedlings of rapeseed in a growth-pouch culture experiment was observed in response to inoculation.	Belimov et al., (2002)
P. putida GR12-2 and an IAA-deficient mutant	*Brassica rapa*, *Vigna radiata*	Primary roots of canola seeds treated with wild-type strain were 35 to 50% longer than the roots from seeds treated with the IAA-deficient mutant and the roots from uninoculated seeds. Exposing mung bean cuttings to high levels of IAA by soaking in a suspension of the wild-type strain stimulated formation of many adventitious roots.	Patten and Glick (2002)
Arthrobacter mysorens 7, *Flavobacterium* sp. *L30*, *Klebsiella mobilis CIAM880*	*Hordeum vulgare*	All the PGPR actively colonized barley root system and rhizosphere, and significantly stimulated root elongation up to 25%.	Pishchik et al., (2002)
B. licheniformis CECT 5106, *B. pumilus* CECT 5105	*Pinus pinea*	Both *Bacillus* strains promoted the growth of *P. pinea* seedlings.	Probanza et al., (2002)
Rhizobium, *Azospirillum*	*Oryza sativa* L.	Inoculation with diazotrophs had significant growth-promoting effects on rice seedlings.	Biswas et al., (2000)
R. leguminosarum (strain E11)	*Oryza sativa* L.	Growth-promoting effects of inoculation on rice seedlings were observed under axenic conditions.	Dazzo et al., (2000)
Azotobacter	*Zea mays* L.	Inoculation with strains efficient in IAA production had significant growth-promoting effects on maize seedlings.	Zahir et al., (2000)
P. putida GR 12-2 (wild type), GR12-2/acd36 (ACC-deaminase minus mutant), GR 12-2/aux1 (IAA-over producers)	*Vigna radiata*	Only the wild-type strain produced longer roots.	Mayak et al., (1999)

Adapted from Khalid et al., (2009)

2.6.1 Response of PSM inoculation to Crops

The advent of P-solubilizing potentials among renewable resources like the microbes has been one of the most important biological traits that have resulted in reducing the dependence on synthetic P fertilizers and consequently preserving soil fertility and environmental safety from chemical toxicity. And therefore, the use of PS bacteria as an alternative to chemical fertilizer has attracted greater attention of agronomists than microbiologists in recent times. In order to develop microphos, organisms with P-solubilizing activity may be isolated from either conventional or derelict environment using standard methods. The isolated bacterial cultures showing greatest P-solubilizing activity are selected and used to develop as microbial inoculants following standard procedure. Subsequently, the microphos are tested both under pot house or field environment using seed treatment, seedling dipping or soil application methods for their ultimate transfer to practitioner/farmers for application in agricultural practices as a cheap and viable phosphatic option. Considering the vast and varied activities, researchers around the world have either attempted or included the use of this novel group of economically feasible biological materials in agronomic operation for sustainable crop production with variable results. The role of PSB in maintaining soil fertility vis-a-vis increasing crop productivity is briefly discussed in the following section.

Realizing the beneficial impact of PSM especially in providing soluble P to plants, many researchers around the world have isolated PS bacteria from different soils (Ahemad and Khan 2010b; Hui et al., 2011; Xiang et al., 2011) and tested their ability as inoculants to see whether they have any impact on plant growth or not (Zaidi et al., 2003; Kumari et al., 2009; Zaidi et al., 2009; Khan et al., 2013). Interestingly, among microbiological option, many of PS bacteria belonging largely to the genera pseudomonads (Behbahani 2010), bacilli (Wani et al., 2007c; Sanjotha et al., 2011; Yadav et al., 2011), rhizobia (Abd-Alla 1994; Abril et al., 2007; Chandra et al., 2007; Marra et al., 2011) and *Azotobacter* (Ivanova et al., 2006; Yi et al., 2008) etc. when used as phosphatic inoculants has been found effective and more practical in sustainable agricultural practices for enhancing crop production by providing available forms of P to different plants (Bojinova et al., 2008; Adesemoye and Kloepper 2009; Oliveira et al., 2009; Yu et al., 2011) in different agro-ecological niches (Khan et al., 2007). In addition to P, the PSM (s) including bacteria (Zaidi et al. 2009; Zhu et al. 2011) and fungi (El-Azouni 2008; Khan et al., 2010) increase the growth of

plants by other mechanisms like, N_2 fixation, and by providing various growth regulating substances to plants (Mittal et al., 2008; Ahemad and Khan 2011b), such as siderophores (Oves et al., 2009; Ahemad and Khan 2012) and antibiotics (Lipping et al., 2008; Khan et al., 2009), and protecting plants from pathogens damage (Hamdali et al., 2008). Documented results have shown that microphos (microbial cultures with PS activity) having such a vast and varied activities when used either alone (Chen et al., 2008; Poonguzhali et al. 2008) or as mixture with other PGPR, a modifier of soil fertility and facilitator of plant establishment (Vikram and Hamzehzarghani 2008; Khan et al., 2009) increased the biological and chemical characteristics of plants grown in various agro-ecosystems (Rodriguez et al., 2006; Ahemad and Khan 2011b). A detailed response of sole and composite PSM inoculation to different crops is highlighted in Table 8.

Table 8. **Examples of sole and composite inoculation effects of phosphate solubilising bacteria on biological and chemical characteristics of different plants**

Organisms applied		Crop	Plant attributes	Reference
Sole	Composite			
P. agglomerans NBRISRM		Maize, Chickpea	Shoot length, leaves, seed, N,P and K uptake	Mishra et al., (2011)
P. chlororaphis, P. fluorescens, B. cereus		Walnut	Plant height, root and shoot dry weight, P, N and K uptake	Yu et al., (2011)
P. fluorescens, P. putida		Wheat	Plant height, tillers, number of grains/spike, 1,000-grain weight, grain and straw yield, N,P and K uptake	Zahibi et al., (2011)
Enterobacter sp		Cowpea	Root and shoot length, dry biomass, seedling length	Deepa et al., (2011)
P. fluorescens, Pantoea		peanut	Plant length, Dry weight, N and P content	Taurian et al., (2010)
P. aeruginosa		Greengram	Plant height, plant dry weight, nodulation, chlorophyll, leghemoglobin, N and P content, seed yield	Ahemad and Khan (2010b)
Citrobacter, Pantoea, Klebsiella and *Enterobacter*		Pigeon pea	Shoot P content, dry shoot/root ratio, dry weieht	Patel et al., (2010)
Bacillus sp.		Chickpea	Root and shoot length, nodulation, dry weight	Wani and Khan (2010)
Burkholderia gladioli, Enterobacter aerogenes and *Serratia marcescens*		*Stevia rebaudiana*	Shoot and root length, leaf and stem dry weight, shoot biomass, and glycoside contents	Mamta et al., (2010)

A.calcoaceticus SE370		Cucumber, Chinese cabbage and Crown daisy	Shoot length, plant height, dry weight	Kang et al., (2009)
Pseudomonas aeruginosa	*Sinorhizobium meliloti*	Mustard	Root and shoot fresh weight and dry weight, Yield	Maheshwari et al., (2011)
Pontibacter niistensis		Cowpea	Root and shoot weight, dry weight, seedling growth	Dastager et al., (2011)
P. fluorescens	*Burkholderia cepcia, Aeromonas vaga*	Mungbean	Root and shoot length, dry weight, leaf area, photosynthetic yield, P content in leaf	Jha et al., (2011)
Pseudomonas	*Bacillus*	Strawberry	Fruit yield and weight, Vit C, reducing sugar	Esitken et al., (2010)
Bacillus, Pseudomonas	*Sinorhizobium meliloti*	Alfalfa	Root and shoot dry weight, root length, Ncontent in shoot	Guiñazú et al., (2010)
Paenibacillus alvei	*Bacillus simplex, Bacillus cereus*	Wheat	Shoot and root biomass and total root length	Hassen and Labuschagne (2010)
Bacillus megaterium	*Bacillus simplex, Bacillus cereus*	Tomato	Shoot and root biomass and total root length	Hassen and Labuschagne (2010)
P. putida	*B. japonicum*	Soybean	Root and shoot dry weight, nodulation	Rosas et al., (2006)
P. putida	*S. meliloti*	Alfalfa	Root and shoot dry weight, nodulation	Rosas et al., (2006)

2.6.1.1 Phosphate solubilizers-legume interactions: Current perspective

The sole or composite application of PSB for raising legume production has received considerable attention worldwide and is reviewed and discussed in the following section.

2.6.1.1.1 Impact of monoculture of PSB on legume improvement

Phosphate solubilizing fluorescent pseudomonads isolated from the groundnut rhizosphere, when used as phosphatic biofertilizer against groundnut plants, enhanced germination by 30% while it increased grain yield by 77%. To test the biocontrol potential of this PSB strain, a plant pathogen *Macrophomina phaseolina* alone was also included, which however decreased the grain yield substantially by 57%. The increase in the yield of ground following PSB application however suggested that *Pseudomonas* strains used in this study had two basic traits- (i) pseudomonads acted as biocontrol agent against *M. phaseolina*, and (ii) that they provided available form of P and consequently enhanced the yield of groundnut (Shweta et al., 2008). Dey et al. (2004) in yet another study observed a significantly higher pod yields, haulm yield and nodule dry weight in PSB (*P. fluorescens)* inoculated peanut plants compared to

those recorded for un-inoculated plants grown in pots and field trials. The seed bacterization also resulted in higher N and P contents in soil. In addition, the pod yields were increased by 23-26%, other plant characteristics such as root length, pod number, 100-kernel mass, shelling out-turns and nodule numbers were also increased following bacterial inoculation. Seed treatment with *P. fluorescens* also depressed incidence of soil-borne fungal diseases, like, collar rot and charcoal rot of peanut (Bhatia et al., 2008) caused by *A. niger*. While considering the overall improvement in inoculated peanut, it was inferred that the increase was due to-(i) the synthesis of IAA, ACC-deaminase and siderophore and (ii) antifungal activity against various fungal pathogens. Similar increase in the biological and chemical characteristics and quality of pea and chickpea under both controlled conditions and field environment following P-solubilizing, auxin, ACC deaminase, ammonia, and siderophore producing strains of *Acinetobacter rhizosphaerae* and *Mesorhizobium mediterraneum* (PECA21) have been reported (Peix et al., 2001; Gull et al., 2004; Gulati et al,. 2009). Likewise, inoculation of greengram seeds with PSB demonstrated a extensive nodulation, and increased shoot dry matter and total dry matter, P-content and P uptake in greengram plants 45 days after sowing relative either to rock phosphate (RP) or single super phosphate (SSP) application (Vikram and Hamzehzarghani 2008).

2.6.1.1.2 Synergistic effect of phosphate solubilizing bacteria with other PGPR/AM-fungi

The beneficial microbes involved in P solubilization in addition to P can also enhance plant growth by improving the efficiency of BNF, accelerating the availability of other trace elements and by production of phytohormones (Wani et al., 2007c). Accordingly, increase in yield of various legumes have been observed following seed or soil inoculation with N_2 fixing organisms, PSB or PSB when used with nodule bacteria (Maheshwari et al., 2011) and/AM-fungus (Zaidi and Khan 2006; Khan and Zaidi 2007). Like other PGPR, PSB within soil forms a close relationship with microbes and play important role in improving crop yields additively or synergistically. For example, the composite application of N_2 fixing *S. meliloti* and P-solubilizing bacterium *Bacillus* sp. M7c and *Pseudomonas* sp. FM7d significantly enhanced the N fixing efficiency of alfalfa plants. Of these, *Pseudomonas* sp. FM7d resulted in enhanced dry matters production in plant organs such as root and shoot, length, and surface area of roots, number, and symbiotic properties of alfalfa plants (Guiñazú et al., 2010). It was concluded from this study that *S. meliloti* B399 and

Bacillus sp. M7c proved effective for developing mixed phosphatic inoculants. In a similar experiment, Bansal (2009) observed a dramatic increase in nodulation and grain yield of mungbean treated simultaneously with *Rhizobium*, PGPR and PSB. The tripartite treatments were followed by dual inoculation of *Rhizobium* with PGPR and *Rhizobium* alone in terms of nodulation and grain yield increases in kharif seasons. The pooled analysis also gave significantly highest number of nodules/plant (21/plant), dry weight of nodules/plant (87.7 mg) and grain yield (12.9 q/ha) following combined inoculation of *Rhizobium*, PGPR and PSB. The increase in yield (12 q/ha) was at par with *Rhizobium* used with PGPR. In a follow up study, Dutta and Bandyopadhyay (2009) while conducting a field experiment during the winter seasons, observed that P and bio-fertilizers, phosphobacterin (*Pseudomonas striata*) and co-inoculation of *Rhizobium* with phosphobacterin, when applied together enhanced the early vegetative growth, symbiotic properties like, nodule production and excessive synthesis of leghaemoglobin in nodules, nitrogenase activity (NA) and yield components such as seed yields, harvest index (HI) and P uptake by chickpea cultivar Mahamaya-2 plants grown in entisol (laterite soil) under rainfed conditions. Of the various combination treatments, seed inoculation of phosphobacterin with *Rhizobium* was significantly better than that of rest of the treatments. When P (26.2 kg/ha) was also added to the mixture of *Rhizobium* and phosphobacterin, the biological and chemical properties of chickpeas were further improved relative to other levels of P used with bio-fertilizer. In other parts of the world like in Erzurum (29° 55' N and 41° 16' E with an altitude of 1950 m), Turkey, a similar investigation was carried out by Elkoca et al., (2008) where they used *Rhizobium*, N_2-fixing *B. subtilis* (OSU-142) and P-solubilizing *B. megaterium* (M-3) to inoculate chickpea plants. Under the field trials, single, dual, and triple inoculations with *Rhizobium*, OSU-142, and M-3 significantly increased plant height, shoot, root and nodule dry weight, N%, chlorophyll content, pod numbers, seed yield, total biomass yield, and seed protein content compared with the control treatment, equal to or higher than N, P, and NP treatments. Interestingly, the mixture containing *Rhizobium* was comparatively better in terms of nodulation than the sole application of *Rhizobium*. Increase in the seed yield under different inoculation treatments ranged between 18 (*Rhizobium*) and 31% (*Rhizobium* with OSU-142 and M-3) over the control whereas N, P, and NP applications corresponds to an increase of 27, 11 and 33%, respectively.

Dual and triple inoculations in general were more effective than other treatments which could probably be due to P activity of *Enterobacter*.

Co-inoculation with PSM and AMF of soils with high phosphate fixation capacity may overcome the limitation mentioned on the effectiveness of PSMs in enhancing plant P uptake. First, mycorrhizal plants can release higher amounts of carbonaceous substance in to rhizosphere (Linderman 1988) than non mycorrhizal plants. Rhizosphere PSM can use these carbon substrates for their metabolic process, which are responsible for organic acid production in the rhizosphere and/or protein excretion. Second, the extensive mycorrhizal network formed around roots can efficiently take up P released by PSMs thus minimizing its re-fixation. Barea et al., (2002) reported that the combined inoculation with PSB, mycorrhizal fungi and *Rhizobium* increased the P uptake by several legumes fertilized with rock phosphate. Mycorrhizal interaction with PSM has been found beneficial and has shown dramatic improvement in plant P uptake in highly weathered soil in contrast to the results obtained for less-weathered soils. Osorio (2011) in his experiments while using PSMs alone and in combination with mycorrhizal fungi in order to assess their impact on growth of *Leucaena leucocephala* found that the overall growth of test plant was highly dependent on the nature of P sorption capacity of soil. The sole application of PSM significantly increased plant growth of *Leucaena* in low P sorption soil while in high P sorption soil mixture of PSM and AMF was significantly greater than single application of PSM. This finding suggested that the effectiveness of PSMs in increasing plant P uptake and growth is controlled by the P sorption capacity. In soils with low P sorption ($P_{0.3}$<100) capacity, though PSM inoculation alone can increase plant growth but in soils with medium and high P sorption (100 < $P_{0.2}$< 500< $P_{0.2}$), PSM alone are less effective or even ineffective, their effectiveness depends on the presence of mycorrhizal association. In other study, Osorio (2008) observed that PSM could desorb P from mineral and soil samples, but this was controlled by the P desorption (higher P desorption at low $P_{0.2}$ value). For minerals, the magnitude on which P desorbed was in the order: montmorillonite> kallonite>goethite>allophone (null description) consequently for soils the order was: mollisol>oxisol>ultisol>andisol. The amount of P desorbed by the PSM was higher when the minerals or soils had higher levels of sorbed P, this is when saturation of sorption sites was higher.

In addition to the PGPR, PSB has been found to form symbiotic relationship with AM- fungi (Wang et al., 2011). For example, Toro et al., (2008) conducted an experiment to test the efficacy of composite microbial inoculations such as a wild type (WT) *R. meliloti* strain, its genetically modified (GM) derivative, the AM-fungus *G. mosseae* (Nicol and Gerd) Gerd and Trappe, and a PSB *Enterobacter* sp. and rock phosphate (RP) on N and P acquisition by alfalfa plants. Interestingly, all the microbial cultures were established well within root tissues and/or in the alfalfa rhizosphere and had no antagonistic effect towards each other. Also, the population of PSB was stimulated following both AM colonization and RP application and GM *Rhizobium* application. Subsequently, there was tremendous improvement in N and P accumulation in alfalfa plants following composite microbial inoculations. Even-though, the *Enterobacter* application had no noticeable effect on N or P accumulation in soil treated with RP but it showed an obvious effect in the non RP-amended controls. In addition, ^{15}N:^{14}N ratio in plant shoots indicated enhanced N_2 fixation rates in *Rhizobium*-inoculated AM-plants, compared to those obtained by the same *Rhizobium* strain in non-mycorrhizal plants. Regardless of the *Rhizobium* strain and of whether or not RP was added, AM-inoculated plants showed a lower specific activity (^{32}P:^{31}P) than did their comparable non-mycorrhizal controls suggesting that the plant was using otherwise unavailable P sources. The P-solubilizing, AM-associated, microbiota could in fact release P ions, either from the added RP or from the indigenous 'less-available' P. Additionally, the proportion of plant P derived either from the labelled soil P (labile P pool) or from RP was similar for AM inoculated and non-mycorrhizal controls (without *Enterobacter* inoculation) for each *Rhizobium* strain, but the total P uptake, regardless of the P source, was far higher in AM-plants which could probably be due to P mobilization by AM-fungi.

2.6.1.1.3 Some examples of Inoculation Effects of Phosphate Solubilizers on Cereal Crops

The use of PSB in agricultural practices dates back to 1950s when some Russian and European scientists applied *Megatierium viphosphateum*, which later on was identified as *Bacillus megatierium* var. *phosphaticum.* The preparation of this bacterium was subsequently called as phosphobacterin (Cooper 1959; Menkina 1963) and when this was used, increased crop yields from 0-70% in Soviet soils. However, similar experiments conducted in United States, failed to produce any significant effect (Smith et al., 1961). Despite conflicting reports on the performance of PSB in

variable agro-ecosystem against a multitude of crops (Yarzábal 2010) they have since been applied and have shown promising results in some parts of the world (Chesti and Ali 2007; Baig et al., 2011). For example, in a trial conducted under both pot and field environments, the biomass and total P of winter wheat (*Triticum aestivum*) was significantly increased following sole application of *Phosphobacterium* strain 9320-SD. However, there was no significant difference in height of the test plants (Chen et al., 2006). Similarly, PSB isolated from stressed environment such as cold temperature region contained *Serratia marcescens* with inherent PGP traits such as IAA, HCN and siderophore production, profoundly enhanced the plant biomass and nutrient uptake of wheat seedlings when grown in cold environment (Selvakumar et al., 2008). In a follow up study, wheat plants inoculated with ACC deaminase secreting PSB, *P. fluorescens* and *P. fluorescens* biotype F, had higher growth, yield, and nutrient use efficiency, when grown in soil treated simultaneously with varying levels of three major nutrients like N, P, and K (at 0, 25, 50, 75, and 100% of recommended doses). However, the overall growth of inoculated wheat plants decreased both under pot and field trials with increasing concentration of synthetic fertilizers. Hence, in most of the cases, significant negative linear correlations were recorded between percentage increases in growth and yield parameters of even inoculated wheat plants. The decline in growth and yield of bacterized wheat plants when grown with increasing chemical fertilizers however raised certain questions. For example, does the rates of fertilizers greater than recommended ones has any direct impact on composition and functional activities of bacteria or excessive rates has any inhibitory effect on plants metabolism? In this context, it is speculated that low fertilizer application, cause reduction in the ACC deaminase activity of PS strains and thereby leads to reduction in the synthesis of stress (nutrient)-induced inhibitory levels of ethylene in the roots through ACC hydrolysis into NH_3 and α-ketobutyrate. Based on this finding it was study suggested that Pseudomonads could be used in combination with appropriate doses of fertilizers for better plant growth and savings of fertilizers (Shaharoona et al., 2008) as also observed by Kumar et al., (2009) and Maheshwari et al., (2011). Such increase in cereal production following PSB such as *P. fluorescens* 153, *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 application has been attributed to both PSA of PSB and their ability to synthesize growth promoting substances (such as ACC deaminase and IAA-like products) in natural soil ecosystem (Zabihi et al., 2011). Interestingly, *P. putida* 108 among the bacterial cultures

displayed enhanced P uptake (96 and 80%) and grain yield (58 and 37%) in wheat under greenhouse and field conditions, respectively. Even though this finding suggested that *Pseudomonas* sp. could serve as an alternative to expensive P application in wheat production system but the better results can be achieved when a compatible bioinoculant is added as mixture with 50% (25 kg/ha P_2O_5) P fertilization. In a recent follow up study, Abbasi et al., (2011) isolated eight PGPR strains, assessed their morphological and cultural characteristics, PSA, and their ability to secrete IAA. Invariably all strains produced IAA (ranging from 5.5–31.0 mg/ml) while only four of them showed P-solubilizing traits. Subsequently, strains WPR-32, WPR-42, and WPR-51 grouped under PGPR category were used both as single and co-culture along with two levels (50 and 100 kg N/ha) of N to evaluate their effect against wheat under greenhouse conditions. As expected, application of PGPR resulted in significant increase in plant height (25%), shoot fresh weight (45%) and shoot dry weight (86%), while it was 27, 102, and 76%, increase in root length, root fresh and dry weight, respectively, over un-inoculated plants. In addition, the number of tillers per plant, 1000-grain weight and grain yield were enhanced by 23, 48 and 59%, respectively over control. The nutrient (N and P) uptake by plant organs like shoot was increased three-folds, while K uptake was increased by 58% following PGPR application. However, the growth, yields and nutrient uptake were increased even further when bacterial cultures were used together with varying levels of N. Apart from the direct effect of PGPR on wheat plants, the concentration of NO^{-3}, N and available P in soil also increased with PGPR application. Moreover, of the varying treatments, mixed bacterial cultures showed better efficiency than the individual ones suggesting that there is no reason to doubt why application of PGPR with N fertilizer cannot increase N contents and N uptake by plants. Also, application of PGPR even with low fertilizer rates could be a more viable option for achieving optimum benefits while reducing the dependence on chemical inputs (Kumar et al., 2009). An interactive and positive effect of PSB, N_2 fixer and AM fungi on plant vigor, nutrient uptake, and yield in wheat plants was observed following composite application of *P. striata* + *A. chroococcum* + *G. fasciculatum*. The available P contents in soil enhanced significantly due to triple inoculation of *A. chroococcum, P. striata*, and *G. fasciculatum*. The residual N content of soil however did not change appreciably even among the treatments. The density of *A. chroococcum*, PSB,

percentage root infection, and spore density of the AM fungus in inoculated treatments increased at 80 days of wheat growth (Zaidi and Khan 2005).

Inoculation of *Burkholderia vietnamiensis* to rice cultivars in two pot and four field trials at different locations of Vietnam, showed an enhancement of 33, 57, 30 and 13% in shoot weight, root weight, leaf area and number of tillers/hill, respectively, compared to non-inoculated plants. In other study, strain of *Rhodobacter capsulatus* significantly increased the plant dry weight, number of productive tillers, grain and straw yields of rice var. Giza 176, grown in pot treated with different levels of N fertilizer compared to non-inoculated plants (Elbadry et al., 1999). The results of this study concluded that N fertilizer could be saved upto 50% while applying bacterial fertilizers. Similarly, an increase of 41, 12, 11.2-20 and 18.7% in root weight, straw yield, grain yield and total biomass, respectively due to PGPR inoculation over non-inoculated rice is reported (Mehnaz et al., 1998; Sherchand 2000). The liquid culture (for pot experiments) or carrier based preparation (for field trials) of three bacterial species such as *B. megaterium*, *B. subtilis* and *P. corrugata*, isolated from temperate locations in the Indian Himalayan region and exhibiting phosphate solubilizing activity (PSA) in the order: *P. corrugata*> *B. megaterium*>*B. subtilis,* when tested caused a dramatic increase in overall performance of rice. While comparing the effect of three cultures, *B. subtilis* had the most promising effect and increased the grain yield by 1.7 and 1.6 fold in pot and field trials, respectively (Trivedi et al., 2007).

Similar variable effects of PSB on other cereals used either alone or in combination with other chemical fertilizers have been reported (Panhwar et al., 2011; Yazdani et al., 2011). For example, like wheat and cereals, there has also been a substantial increase in the biomass of maize plants inoculated with *S. marcescens* (EB 67) and *Pseudomonas* sp. (CDB 35) (Hameeda et al., 2008). In this experiment, strain EB 67 enhanced the dry matter accumulation by 99% while it was 94% by strain CDB 35. Grain yield of inoculated maize increased by 85% and 64%, following EB 67 and CDB 35 application, respectively. When applied as mixture with arbuscular mycorrhizal (AM) fungi *Glomus intraradices,* the PSB *Pseudomonas fluorescens* had a positive impact on plant growth, nutrient uptake, grain yield and yield components in maize plants. Composite inoculation of the two cultures significantly increased grain yield, yield components, harvest index, grain N and P, soil available P, root colonization percentage under water stress conditions. However, some of the assayed characteristics under well-watered conditions were non- significantly higher in

chemical fertilizer treatment compared to those observed for dual inoculation treatments. However, the effect of sole application of *P. fluorescens* (Pf) was poor relative to the composite application of AM fungus with PSB or single application of AM fungi. The measured parameters of inoculated plants were in general higher than uninoculated plants under water deficit stress conditions. In addition, the characteristics determined for co-inoculated plants grown under severe water stressed conditions were significantly lower than co-inoculated plants grown under well-watered and moderate-stressed conditions. This finding suggested that PSB can interact positively with other organism like AM fungi as observed in this study and can be used to facilitate plant growth and P uptake by maize plants, leading to plant tolerance improving under water deficit stress conditions (Ehteshami et al., 2007). In a recent study, Rajapaksha et al., (2011) conducted experiments under both pot and field environment to assess the substitutability of triple superphosphate (TSP) by a P fertilizer mixture (PFM) involving TSP, RP, and PSB inoculants for wetland rice. For these studies, 6 single and 2 dual inoculants were formulated with *Enterobacter gegovie* and 5 *Bacillus* species. In pot trials, the mixture of *E. gergovie* and *B. mycoides* and the sole application of *B. subtilis* enhanced yields by 32 and 25%, respectively, relative to single application of TSP. The results observed in pot trials were validated under field environment where dual culture of *E. gegovie* with *B. subtilis,* and *E. gergovie* with *B. pumilus* augmented grain yield by 22–27% compared to TSP application alone (574 gm^{-2}). Overall, it was suggested that about 50% of TSP could be saved when RP is applied with *E. gegovie, B. pumilus,* and *B. subtilis,* as seed inoculant for raising the productivity of rice both under pot and field conditions.

3. 1 Collection of soil samples and microbial diversity

The soil samples were collected from the rhizospheric soils of chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), greengram [*Vigna radiata* (L.) Wiclzek), lentil (*Lens esculentus*), mentha (*Mentha avensis*), chilli (*Caspsicum annuum*), cabbage (*Brassica oleracea* var *capitata*) and mustard (*Brassica compestris*) grown during 2009-2010 season at the experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Three soil samples were collected from each rhizosphere in sterilized polythene bags (15×12 cm^2). The soil samples were mixed thoroughly and were used for determining microbial diversity. The total bacterial, fungal, actinomycetal populations, phosphate solubilizing microorganisms (PSM) and asymbiotic nitrogen fixer (*Azotobacter*) were isolated using standard media and microbiological methods (Holt et al., 1994). For this, soil samples were serially diluted in sterile normal saline solutions (NSS) (Appendix 1) and 100 µl of diluted suspension was spread plated (Buck and Cleverdon 1960) on nutrient agar (Appendix 2), Martin's medium (Appendix 3), Kenknight's medium (Appendix 4), Pikovskaya (Pikovskaya, 1948) medium (Appendix 5) and Ashby's medium (Appendix 6) for total bacterial counts, fungal populations, actinomycetes, phosphate solubilizers and *Azotobacter*, respectively. Each sample was replicated three times and incubated at 28±2 °C for two (bacteria), three (fungi), five (actinomycetes) and five to seven (PSM and *Azotobacter*) days. Where microbiological assay was not done immediately, the soil samples were kept in sterile polythene bags and stored at 4 °C for a short period of time. Standard culture medium and growth conditions used for isolation and enumeration of microbial populations are given in Table 9.

3.2.1 Isolation of symbiotic and asymbiotic nitrogen fixers

The symbiotic nitrogen fixing rhizobia were isolated by spread plate method using yeast extract mannitol agar (YEMA) (Appendix 7) from the fresh nodules of chickpea, pea, greengram and lentil (Table) grown in the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, U.P., India, using standard method (Somasegaran and Hoben 1985). Briefly, the nodules were removed carefully from the root systems of each healthy legume plants and surface sterilized by dipping nodules in 2.5% sodium hypochlorite for two minutes. Nodules were then rinsed in 95% ethanol (v/v) and washed six times with sterile water and squashed in normal saline solution. Nodule suspensions were then diluted in NSS and 100 µl of each

suspension was spread plated on YEMA medium containing 2.5% Congo- red as indicator. Inoculated plates were incubated at 28±2 °C for three to five days. A single colony was picked and streaked four times on the YEMA plate to check the purity of the isolated cultures. Rhizobial colonies were maintained on the YEMA slants at 4 °C until use.

3.2.2 Isolation and screening of phosphate solubilizing bacteria

The phosphate solubilizing bacteria (PSB) were isolated from rhizospheric soils of mentha, chilli, cabbage and mustard grown in the experimental fields of Faculty of Agricultural Sciences, AM U, Aligarh by spread plate method using Pikovskaya agar medium. A 100 µl of serially diluted suspension of each rhizospheric soil sample was spread plated on solid Pikovskaya medium and plates were incubated for seven days at 28±2 °C. Bacterial colonies forming clear halo (zone of solubilization) on solid Pikovskaya plates within seven days were considered as phosphate solubilizers. The phosphate solubilizers were maintained on Pikovskaya medium until use.

Table 9-Culture medium and growth conditions used for isolation and enumeration of microbial populations

Microbes	Medium	Incubation temperature (°C)	pH of medium	Incubation period (days)
Bacteria	Nutrient agar	28±2	7±0.2	1-2
Fungi	Martin's agar	28±2	7±0.2	3-5
Actinomycetes	Kenknight's agar	28±2	7±0.2	5-7
PSM	Pikovskaya agar	28±2	7±0.2	5-7
Azotobacter spp.	Ashby's agar	28±2	7±0.2	5-7
Rhizobia	Yeast extract mannitol agar	28±2	7±0.2	2-5

3.3 Identification of the potential plant growth promoting rhizobacterial strains

The plant growth promoting rhizobacteria including phosphate solubilizers and nitrogen fixers were identified using morphological such as colony morphology, Gram reaction and shape of microbes, while, biochemical tests included indole reaction, citrate utilization test, methyl red test, Voges-Proskauer, catalase, oxidase test, starch, gelatin, lipid hydrolysis, mannitol salt utilization test and sugar fermentation test. The test employed for characterization and identification of bacterial isolates are discussed briefly in the following reaction.

3.3.1 Morphological characteristics

A total of 100 isolates including N_2 fixer (N=70) and P-solubilizing bacteria (N=30) were selected and characterized morphologically and biochemically.

3.3.1.1 Colony morphology: Colony morphology of the isolated bacterial cultures included the certain features such as shape, margin (serrated or smooth), color, watery or mucoid colony.

3.3.1.2 Gram reaction: Cultures were Gram stained (Appendix 8). Bacteria showing purple color were grouped as Gram positive and those showing pink color were grouped as Gram negative.

3.3.2Biochemical properties

3.3.2.1 Indole reaction: Using autoclaved nutrient broth (Appendix 9), each test isolate was incubated at 28±2 ^{0}C for 24-48 h. After incubation, 2-3 drops of Kovac's reagent (Appendix 10) was added to broth and observed for the formation of red ring.

3.3.2.2 Citrate utilization test

Autoclaved Simmon's citrate agar (Appendix 11) plates were spot inoculated with test isolates and incubated at 28±2 ^{0}C for 24-48 h. Change in color from green to blue was observed.

3.3.2.3 Methyl red test: Autoclaved MR-VP broth (Appendix 12) inoculated with each isolate was incubated at 28±30 ^{0}C for 24-48 h. Methyl red solution (Appendix 13) was added as indicator. The development of red color was observed.

3.3.2.4 Nitrate reduction test: Autoclaved trypticase nitrate broth (Appendix 14) tubes inoculated with test isolates were incubated at 28±2 ^{0}C for 24-48 h. Five drops of solution A and few drops of solution B were added and examined for formation of red color.

3.3.2.5 Voges-Proskaeur test: Autoclaved MR-VP broth was inoculated with test organism and incubated at 28±2 ^{0}C for 24-48 h. After incubation, Barrit's reagent (Appendix 15) was added and observed for red color formation.

3.3.2.6 Catalase test: Test isolates were inoculated in nutrient broth and incubated at 28 ±2 $^\circ$C for 24-48 h. A 3%, H_2O_2 was added and observed for bubble formation.

3.3.2.7 Oxidase test: Oxidase disc was moistened with distilled water. Cultures were spread on plates containing oxidase disc. The color of the disc changed from deep blue to deep purple indicating a positive test, while the colorless spot indicated the negative test.

3.3.2.8 Starch hydrolysis test: Autoclaved starch agar plates (Appendix 16) were spot inculcated with test isolates and incubated at 28±2 °C for 24-48 h. After incubation, plates were flooded with iodine solution. Clear zone of hydrolysis around the growth was observed.

3.3.2.9 Gelatin hydrolysis: Tubes containing autoclaved nutrient broth, amended with 12% gelatin tubes were inoculated with test isolates and incubated at 28±2 °C for 48 h. After incubation, tubes were placed at 4 ^{0}C for 30 min. On refrigeration, liquefied tubes indicated positive test.

3.3.2.10 Lipid hydrolysis test: For this, test cultures were spot inoculated on tributyrin agar (Appendix 17) and were incubated at 28±2 °C for 24-48 h. After incubation, clear zone of lipolysis surrounding the bacterial growth was observed.

3.3.2.11 Mannitol salt utilization: Each test organism was spot inoculated on autoclaved mannitol salt agar plates (Appendix 18) and incubated at 28±30 °C for 24-48 h. Change in color from red to yellow was observed.

3.3.2.12 Sugar fermentation test: Autoclaved fermentation broth (Appendix 19) supplemented with 5 g/l each of sucrose, lactose, fructose, glucose, arabinose, xylose, mannose and inositiol was inoculated with test isolates and incubated at 28±2 °C for 24-48 h. Production of acid alone or acid with gas was observed.

3.4 Antibiotic sensitivity behaviour of isolated cultures

Antibiotic sensitivity behaviour was determined by the using the antibiotic discs of known potency (Table 10) by disc diffusion method of Bauer et al., (1966). Freshly prepared and autoclaved nutrient broth was inoculated by isolated bacterial cultures and incubated for 24 h at 28±2 °C. A- 100 µl of overnight grown test cultures was taken on plates and was evenly spread with sterile glass rod spreader. Plates were then mounted with individual antibiotics disc using a sterile forceps. Each antibiotics mounted plate was incubated at 28±2 °C for 24-48 h. After incubation, zone of inhibition was measured and the strains were scored as resistant (R) and susceptible (S). Following the standard antibiotics disc sensitivity testing method (Margalejo et al., 1984), the plates were recorded for comparing the zone of inhibition (diameter in mm) with chart provided by the manufacturers.

Table 10- Antibiotics and their potency used in the present study

S.No.	Antibiotics disc	Disc code	Disc potency (µg/disc)
1.	Amoxycillin	Am	30
2.	Chloramphenicol	C	25
3.	Ciprofloxacin	Cf	30
4.	Cloxacillin	Cx	30
5.	Doxycillin hydrochloride	Do	5
6.	Erythromycin	E	10
7.	Gentamycin	G	30
8.	Kanamycin	K	30
9.	Methicillin	M	30
10.	Nalidixic acid	NA	30
11	Nitrofurantoin	Nf	30
12.	Norfloxacin	Nx	10
13	Novobiocin	Nv	30
14.	Penicillin G	P	10
15	Polymixin B	Pb	50
16	Rifampicin	R	30
17	Tetracycline	T	30

Source: Hi-media Pvt. Ltd., Mumbai, India

3.5 Screening of bacterial strain for 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity

Using the spot inoculation method, five micro litre of each isolated rhizobial (N=50) strains and phosphate solubilizing bacteria (N=30) was placed on a section of plate (marked in 16 equal parts) containing DF (Dworkin and Foster 1958) salt minimal medium (Appendix 20) supplemented with three mM ACC instead of $[(NH_4)_2SO_4)]$ and incubated at 28±2 °C for 72 h and the growth was checked daily as suggested by Penrose and Glick (2003). *Mesorhizobium* LMS-1 containing pRKACC plasmid was used as ACC deaminase positive control in this study (Nascimento et al., 2011b). All the samples were tested in duplicates and experiments were repeated three times.

3.6.1 Identification based on 16S rRNA sequencing

Of the total 30 P-solubilizers, only eight bacterial strains namely, PSE3, PSE5, ES1, ES2, ES3, ES4, ES5 and ES6 expressing adequate ACC deaminase activity and showing greater P-solubilizing activity *in vitro*, were selected and identified to species level using 16S rRNA gene sequence analysis. For identification, a partial 16S rRNA gene sequences of selected strains was done commercially from Macrogen Inc., Seoul, South Korea, using universal primers, 518F (5'CCAGCAGCCGCGGTAATACG3') and 800R (5'TACCAGGGTATCTAATCC3'). All nucleotide sequence data were

deposited in the GenBank sequence database. The online program BLASTn was used to find related sequences with known taxonomic information in the databank at the NCBI website (www.ncbi.nml.nih.gov/BLAST) to accurately identify and compare the isolates with nearest neighbour sequence available in the NCBI database.

3.6.2 Construction of phylogenetic tree

The sequence obtained from Macrogen were initially estimated by the BLASTn online programme facility of NCBI (www.ncbi.nml.nih.gov/BLAST) and then aligned with all related sequences obtained from GenBank by Cluastal W (Thompson et al., 1994). Phylogenetic tree was reconstructed by neighbour-joining method (Saitou et al., 1987). Bootstrapped neighbour-joining relationships were estimated with MEGA4 software (Tamura et al., 2007).

3.7.1 Quantitative assay of P-solubilization

The bacterial strains expressing phosphate solubilizing activity during screening process was tested further for quantitative estimation of P-solubilized under *in-vitro* conditions. Also the solubilization index (SI) and solubilizing efficiency (SE) of the P-solubilizing organism was calculated by the formula as suggested by Premono et al., (1996) as:-

solubilization index (SI) = (colony diameter + zone of halo)/colony diameter.

solubilizing efficiency SE= (zone of halo/colony diameter)×100

The clear halo around bacterial growth was measured and bacterial cultures were further used to determine the extent of phosphate solubilization in liquid Pikovskaya medium. For the quantitative measurement of P, 100 ml of Pikovskaya broth containing 5g tri-calcium phosphate (TCP) was inoculated with one ml of 10^8 cells/ml of each culture. The flasks were incubated for 5, 10 and 15 days with shaking at 120 rpm at 28±2 °C. A- 20 ml culture broth from each flask was removed and centrifuged (9000 g) for 30 min. and the amount of water soluble P released into the supernatant was estimated by the chlorostannous-reduced molybdophosphoric acid blue method (King, 1932; Jackson, 1967). To 10 ml of supernatant, 10 ml chloromolybdic acid (Appendix 21) and 5 drops of chlorostannous acid (Appendix 22) was added and volume was adjusted to 50 ml with distilled water. The absorbance of blue color developed was read at 600 nm. The amount of P-solubilized was calculated using the calibration curve of KH_2PO_4. The change in pH following TCP solubilization was also recorded. Each independent experiment was repeated three times after several subcultures. SI and SE of the bacterial isolates showing greater solubilization on both

solid and liquid medium and maintaining the PS activity after several subcultures were chosen as the efficient PS strains for further studies.

3.7.2 Quantitative assay of ACC deaminase activity

1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was assayed according to a method of Honma and Shimomura (1978) later modified by Penrose and Glick (2003). According to this method, amount of a-ketobutyrate was measured which is produced by reaction of the enzyme ACC deaminase which cleaves ACC to a-ketobutyrate and NH_3. The number of mmol of α-ketobutyrate produced by this reaction is determined by comparing the absorbance at 540 nm of a sample to a standard curve of a-ketobutyrate ranging between 0.1 and 1.0 mmol. A stock solution of 100 mM α-ketobutyrate (Sigma-Aldrich) was prepared in 0.1M Tris-HCl pH 8.5 and stored at 4 ^{0}C. Just prior to use, the stock solution was diluted with the same buffer to make a 10-mM solution from which a standard concentration curve was generated. Each in a series of known a-ketobutyrate concentrations was prepared in a volume of 200 ml, 300 ml of the 2,4-dinitrophenylhydrazine reagent (0.2% 2,4-dinitrophenyl-hydrazine in 2M HCl) (Sigma-Aldrich) was added, and the contents are vortexed and incubated at 30 $^\circ$C for 30 min. during which time the a-ketobutyrate is derivatized as a phenylhydrazone. The color of the phenylhydrazone is developed by the addition of 2.0 ml 2M NaOH; after mixing, the absorbance of the mixture is measured at 540 nm.

ACC deaminase activity was measured in bacterial extracts prepared in the following manner. All ACC deaminase positive N_2 fixers and P-solubilizers (10^8 cells/ml) were inoculated in YEM broth and Luria Bertani broth, respectively, and incubated in a shaking incubator 200 rpm for 24-48 h at 28±2 $^\circ$C. Then the cultures were centrifuged at 8000g for 10 min at 4 ^{0}C for harvesting the accumulated biomass of N_2 fixers and P-solubilizers. The supernatant was removed and the cells were washed with 5ml DF salts minimal medium. Following an additional centrifugation for 10 min. at 8000g at 4 $^\circ$C, the cells were suspended in 7.5 ml DF salts minimal medium in a fresh culture tube. Just prior to incubation, the frozen 0.5M ACC solution was thawed, and an aliquot of 45 ml was added to the cell suspension to obtain a final ACC concentration of 3.0 mM. The bacterial cells were re- shaken in incubator to induce the activity of ACC deaminase at 200 rpm for 24 h at the same temperature as was done for overnight incubated cultures. The bacteria were harvested by centrifugation at 8000g

for 10 min. at 4 °C. The supernatant was removed, and the cells were washed by re-suspending the cell pellets in 5 ml 0.1M Tris-HCl at pH 7. Each bacterial cell pellet, prepared as described above, were suspended in 1 ml of 0.1M Tris-HCl, pH7.6, and transferred to a 1.5-ml micro-centrifuge tube. The contents of the 1.5-ml micro-centrifuge tube were spun at 16000g for 5 min. and the supernatant was removed. The pellet was suspended in 600 ml 0.1M Tris-HCl, pH 8.5. Thirty microlitres of toluene were added to the cell suspension and vortexed at the highest setting for 30 seconds. At this point, a 100-ml aliquot of the 'toluenized cells' was set aside and stored at 4 °C for protein assay by Lowrey (1951) method at a later time. The remaining toluenized cell suspension was immediately assayed for ACC deaminase activity. All sample measurements were carried out in duplicate. Two hundred microlitres of the toluenized cells were placed in a fresh 1.5-ml micro-centrifuge tube; 20 ml of 0.5 M ACC were added to the suspension, briefly vortexed, and then incubated at 30 ^{0}C for 15 min. Following the addition of 1ml of 0.56 M HCl, the mixture was vortexed and centrifuged for 5 min. at 16000g at room temperature. One millilitre of the supernatant was vortexed together with 800 ml of 0.56 M HCl. Thereupon, 300 ml of the 2,4-dinitrophenylhydrazine reagent (0.2% 2,4- dinitrophenylhydrazine in 2M HCl) was added to the glass tube, the contents were vortexed and then incubated at 30 °C for 30 min. Following the addition and mixing of 2 ml of 2N NaOH, the absorbance of the mixture was measured at 540 nm.

3.7.3 Quantitative assay of indole acetic acid

Indole-3-acetic acid synthesized by bacterial strains was quantitatively evaluated by the method of Gordon and Weber (1951), later modified by Brick et al. (1991). For this, the N_2 fixing and P-solubilizing bacterial strains were grown in Luria Bertani (LB) broth (Appendix 23). Luria Bertani broth (100 ml) having 0, 50, 100, 200, 400 and 500 µg/ml tryptophan was inoculated with one ml culture (10^8 cells/ml) of both N_2 fixing and P-solubilizing bacterial isolates and were incubated for 3, 6, 9 and 12 days at 28±2 °C with shaking at 125 rpm. After incubation, a five mililitre of culture of each treatment was centrifuged (9,000g) for 15 min. and an aliquot of two ml supernatant was mixed with 100 µl of orthophosphoric acid and four mililitre of Salkowsky reagent (2% 0.5M $FeCl_3$ in 35% per-chloric acid) and incubated at 28±2 °C in darkness for 1h. The absorbance of developed pink color was read at 530 nm. The IAA concentration in the supernatant was determined using a calibration curve of

pure IAA as a standard. The experiment was repeated three times on different time intervals.

3.7.4 Qualitative and quantitative estimation of siderophores

The N_2 fixing and P-solubilizing bacterial strains were further tested for siderophore production using Chrome Azurol S (CAS) agar medium (Appendix 24) following the method of Alexander and Zuberer (1991). Chrome Azurol S agar plates were prepared separately and divided into equal sectors and spot inoculated with 10 µl of 10^8 cells/ml and incubated at 28±2 °C for five days. Development of yellow orange halo around the bacterial growth was considered as positive for siderophore synthesis. Each individual experiment was repeated three times. The production of siderophore by the test strains were further detected quantitatively using Modi medium (Appendix 25). Modi medium was inoculated with 10^8 cells/ ml of bacterial cultures and incubated at 28±2 °C for five days. Catechol type phenolates were measured on ethyl acetate extracts of the culture supernatant using a modification of the ferric chloride-ferricyanide reagent of Hathway. Ethyl acetate extracts was prepared by extracting 20 ml of supernatant twice with an equal volume of solvent at pH 2. Hathway's reagent was prepared by adding one milliliter of 0.1 M ferric chloride in 0.1 N HCl to 100 ml of distilled water, and to this, was added one milliliter of 0.1 M potassium ferricyanide (Reeves et al., 1983). For the assay, one volume of the reagent was added to one volume of sample and absorbance was determined at 560 nm for salicylates with sodium salicylate as standard and at 700 nm for dihydroxy phenols with 2, 3-dihydroxy benzoic acid (DHBA) as standard.

3.7.5 Assay of hydrogen cyanide (HCN) and ammonia

Hydrogen cyanide production by bacterial isolates was detected by the method of Bakker and Schipper (1987). For HCN production, N_2 fixing and P-solubilizing bacterial strains were grown on an HCN induction medium (Appendix 26) for 3-4 days at 28±2 °C. For each bacterial isolate, a-100 µl of 10^8 cells/ml was spread on the Petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2% Na_2CO_3 was placed at the lid of the Petri plates. Plates were sealed with parafilm. After four days incubation at 28±2 ^{0}C, an orange brown color of the paper indicating HCN production was observed. For ammonia assessment, the bacterial strains were grown in peptone water (Appendix 27) and incubated at 28±2 °C for four days. One mililitre of Nessler's reagent (Appendix 28) was added to each tube and the

development of yellow color indicating ammonia production was recorded following the method of Dye (1962).

3.7.6 Bioassay of exo-polysaccharides

The exo-polysaccharides (EPS) produced by the bacterial strains was determined under *in vitro* conditions as suggested by Mody et al., (1989). For this, N_2 fixing and P-solubilizing bacterial strains were grown in 100 ml capacity flasks containing basal medium supplemented with 5% sucrose. Inoculated flasks were incubated for five days at 28±2 °C on rotary shaker (100 rpm). Culture broth was spun (5433g) for 30 min. and EPS was extracted by adding three volumes of chilled acetone (CH_3COCH_3) to one volume of supernatant. The precipitated EPS was repeatedly washed three times alternately with distilled water and acetone, transferred to a filter paper and weighed after overnight drying at room temperature.

3.7.7 Determination of antifungal activity

Antifungal activity of isolated rhizobia (N=50), *Azotobacter* (N=20) and PS bacteria (N=30) against plant pathogenic fungi namely, *Rhizoctonia* sp., *Penicillium* sp. and *Alternaria* sp. was assessed on agar plates as described by Weller and Cook (1986) and Wong and Baker (1984). Fungal pathogens maintained on potato dextrose agar (PDA) were transferred to Petri- dishes containing fresh PDA (Appendix 28) to produce fungal mycelium plugs. Rhizobia *Azotobacter* and PS bacteria were grown in yeast extract mannitol broth, Ashby's broth and Luria Bertani broth respectively. A-one ml stationary cell of each bacterial isolate (10^8 cells/ml) was inoculated into 100 ml YEM broth, Ashby's broth and Luria Bertani broth, for rhizobia, *Azotobacter* and PS bacteria respectively. The samples (1.8 ml) of each broth were removed in eppendrof and centrifuged at 3,875×g for 10 min. and the supernatants were filtered through sterile Millipore filter. A- 200 µl sample of each strain was placed in an 8 mm well cut into the centre of pre-inoculated fungal plates. Inoculated plates were incubated at 28±2 °C for 2 days (PS bacteria), 5 days (rhizobia and *Azotobater*) and zone of growth inhibition (mm) was recorded. Each individual experiment was replicated three times at different time intervals.

3.8 Scanning Electron Microscopy

Bacterial cultures belonging to genera *Pseudomonas*, *Bacillus* and *Azotobacter* were grown in LB broth in 250 ml flask on orbital shaker at 28±2 °C. Overnight grown culture of each strain was centrifuged at 4 ^{0}C for 10 min. The cells were washed three times with 0.1 M PBS and fixed overnight in 2% gluteraldehyde (prepared in 0.M

PBS). The cells were washed with PBS and distilled water prior to dehydration through an ethanol series (10% to absolute), held at each concentration for 30 min. Samples were placed on brass stub, sputter-coated with gold then examined by Scanning Electron Microscope (SEM-JEOL-JSM5800LV).

3.9 Pot and field experiments

3.9.1 Seed and planting of legume

Healthy and inoculated/uninoculated seeds of chickpea, pea, greengram and lentil were sown in soils treated with or without recommended rates of nitrogenous and phosphatic fertilizers, as mentioned in Table 11.

Table 11- Rates of urea and DAP used for legumes

Legumes	Cultivar	Fertilizers (kg/ha)	
		Urea	DAP
Chickpea	Avrodhi	30	80
Pea	Arakle	20	90
Green gram	K-851	25	85
Lentil	Malka	30	90

3.9.2 Microbial treatments, fertilizer application and legume growth

Seeds of the commonly grown legumes such as, chickpea, pea, greengram, and lentil were purchased from Prakash Agrochemicals and seeds, Aligarh, U.P., India. Seeds were surface sterilized with 70% ethanol, 3 min.; 3% sodium hypochlorite, 3 min. (Vincent 1970) rinsed six times with sterile water and dried.

Prior to inoculation of seeds with PGPR, the cell suspension of isolate was grown in YEM broth (for rhizobia) and Pikovskaya broth (for phosphate solubilizer) in flasks shaken at 120 rpm at 28±2 °C for five and three days respectively to a cell density of 6x 10^8 (rhizobia) and 3x10^8 cells/ml (for phosphate solubilizers). Surface sterilized seeds were coated separately with ACC deaminase positive plant growth promoting *Pseudomonas putida* strain PSE3, *Bacillus pumilus* strain ES3, *Azototobacter* strain AZ19, *Mesorhizobium* strain RG5, *Rhizobium* strain RP2, *Bradyrhizobium* strain RB6, and *Rhizobium* strain RV9, respectively. While for mixed inocula, equal volume of rhizobial culture and P-solubilizers was mixed (Table 12). Seeds of each legume were soaked in liquid culture medium for 2 h using 10% gum arabic as sticker to deliver approximately 10^8 cells/seed each for rhizobia and *P. putida, B. Pumilus,* and *Azotobacter*. The non-coated sterilized seeds used as control were soaked in sterile water only. The non-inoculated and inoculated seeds (10 seeds per pot) were sown on October 10, 2009 (chickpea), November 1, 2009 (pea), March 15, 2010 (greengram)

and November 7, 2010 (lentil) in clay pots (25 cm high, 22 cm internal diameter) using three kg unsterilized sandy clay loam soil [Silt 190 g/ kg, Clay 143 g/ kg,Organic matter 6.2 g /kg, Kjeldahl N 0.75 g/ kg, Olsen P 16 mg/ kg, pH 7.2 and water holding capacity0.44 ml g^{-1}, Cation exchange capacity 11.7 cmol kg^{-1}, Anion exchange capacity; 5.1 cmol/ kg

Salt conductivity 59.1 μSm^{-1}] and the chemical fertilizer like urea and DAP were added post emergence of each seedling. Each treatment was replicated six times for all crops under study and was arranged in a completely randomized design. Plants in each pot were thinned to three plants 10, 10, 7 and 7 days after emergence (DAE) of chickpea, pea, greengram and lentil respectively. Moreover, for field experiment the non-inoculated and inoculated seeds were sown on October 10, 2010 (chickpea), and November 10, 2010 (pea), March 15, 2011 (greengram) and November 7, 2011 (lentil) in 5×5 m^2 plots treated with or without recommended doses of chemical fertilizers for their respective legumes. The pots were watered with tap water when required and were maintained in open field conditions. All treatments were repeated the following year with the identical environmental conditions to ensure the reproducibility of the results.

Table 12- Experimental design for pot and field trials

Treatments	Legumes			
	Chickpea	**Pea**	**Greengram**	**Lentil**
T1 :Control	NFNI*	NFNI*	NFNI*	NFNI*
T2 :Urea (kg/ha)	30	20	25	30
T3 :DAP (kg/ha)	60	90	85	90
T4 :P-solubilizer	*P. putida, B. pumilus, Azotobacter*	*P. putida, B. pumilus, Azotobacter*	*P. putida, B. pumilus, Azotobacter*	*P. putida, B. pumilus, Azotobacter*
T5 :rhizobia	*M. ciceri*	*R. leguminosarum*	*Bradyrhizobium* sp. (vigna)	*Rhizobium* sp.
T6: P-solubilizer+ Urea	*P. putida*+30 kg/ha *B. pumilus* +30kg/ha *Azotobacter*+30kg/ha	*P. putida*+20kg/ha *B. pumilus* +20kg/ha *Azotobacter*+20kg/ha	*P. putida*+25kg/ha *B. pumilus* +25kg/ha *Azotobacter*+25kg/ha	*P. putida*+30kg/ha *B. pumilus* +30kg/ha *Azotobacter*+30kg/ha
T7: rhizobia+DAP	*M. ciceri* +60 kg/ha	*R. leguminosarum*+90 kg/ha	*Bradyrhizobium* +80 kg/ha	*Rhizobium* +90 kg/ha
T8: rhizobia+ P-solubilizer	*M. ciceri*+ *P. putida* *M. ciceri*+ *B. pumilus* *M. ciceri*+ *Azotobacter*	*R. leguminosarum* + *P. putida* *R. leguminosarum* + *B. pumilus* *R. leguminosarum* + *Azotobacter*	*Bradyrhizobium* + *P. putida* *Bradyrhizobium* + *B. pumilus* *Bradyrhizobium* + *Azotobacter*	*Rhizobium* + *P. putida* *Rhizobium* + *B. pumilus* *Rhizobium* + *Azotobacter*
T9 Urea+ DAP	30 kg/hac+80 kg/ha	20 kg/ha+ 90 kg/ha	25kg/ha+ 85 kg/ha	30 kg/ha+90 kg/ha

*NFNI: represents neither fertilizer nor inoculant

Plant growth such as the length of roots and shoots and dry matter accumulation in roots, shoots and whole plants was recorded at each sampling intervals (Table 13). The remaining three pots for each treatment, having three plants per pot were maintained until harvest. The total N and P content in roots and shoots for all the legume crops were measured at each sampling day by the micro-Kjeldahl method (Iswaran and Marwah, 1980) and the method of Jackson (1967), respectively. The total chlorophyll content in fresh foliage of each legume was quantified at 90 DAS for chickpea, pea and lentil and 50 DAS (greengram) by the method as discussed earlier. The leghaemoglobin content in fresh nodules recovered from the root system of each legume crop was quantified at 90 DAS for chickpea, pea and lentil and 50 DAS for greengram, respectively, by the method as discussed earlier. Seed yield and grain protein (Lowrey 1951) in chickpea, pea, greengram, and lentil were estimated at harvest.

Table 13- Schemes followed for the harvest of plant

Parameters measured	Legumes			
	Chickpea	Pea	Greemgram	Lentil
Root length	90 & 135 DAS	90 & 120 DAS	50 & 80 DAS	90 & 120 DAS
Shoot length	90 & 135 DAS	90 & 120 DAS	50 & 80 DAS	90 & 120 DAS
Dry root biomass	90 & 135 DAS	90 & 120 DAS	50 & 80 DAS	90 & 120 DAS
Dry Shoot biomass	90 & 135 DAS	90 & 120 DAS	50 & 80 DAS	90 & 120 DAS
Chlorophyll content	60 DAS	60 DAS	50 DAS	60 DAS
Nodule no.	90 DAS	90 DAS	50 DAS	90 DAS
Nodule dry biomass	90 DAS	90 DAS	50 DAS	90 DAS
Lb content	90 DAS	90 DAS	50 DAS	90 DAS
P-conetnt	135 DAS	120 DAS	80 DAS	120 DAS
N-content	135 DAS	120 DAS	80 DAS	120 DAS
Seed yield	135 DAS	120 DAS	80 DAS	120 DAS
Seed protein	135 DAS	120 DAS	80 DAS	120 DAS

DAS: days after sowing

Parameters measured

3.9.2.1 Length, biomass production and symbiotic attributes

All plants in three pots for each treatment were removed at 90 and 135 days after sowing (DAS) of chickpea, 90 and 120 DAS for pea and lentil and 50 and 80 DAS for greengram, respectively. The roots were carefully washed and nodules from the root systems of each legume were separated, counted, oven dried at 80 °C and weighed. Plant growth, such as length of roots and shoots, dry weights of root and shoot and total dry plant biomass of all the four legumes was recorded at each sampling dates. Plants uprooted at all the sampling intervals were oven dried at 80 °C to measure the total plant biomass.

3.9.2.2 Quantitative estimation of leghaemoglobin

The leghaemoglobin (Lb) content in fresh nodules recovered from the root system of each legume plants grown in pot trials and fields were quantified at 90 DAS each for chickpea and pea and lentil and 50 DAS for greengram, respectively, by the method of Sadasivam and Manickam, (1992). Fresh nodules were crushed with the help of mortar and pestle in 5 ml sodium phosphate buffer (pH 7.4) and filtered through two layers of cheese cloth. The nodule debris was discarded. The turbid reddish brown filtrate was clarified by centrifugation at 10000 g for 30 min. The supernatant was diluted to 10 ml with sodium phosphate buffer (pH 7.4) (Appendix 30). The extract was divided equally into two glass tubes (5 ml /tube) and equal amount of alkaline pyridine reagent (Appendix 31) was added to each tube. The haemochrome formed was read at 556 and 539 nm after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively. The leghaemoglobin content was calculated using the formula –

$$\textbf{Lb content (mM)} = \frac{[A_{556} - A_{539}] \times 2D}{23.4}$$

Where D= Initial dilution

3.9.2.3 Total chlorophyll content

The total chlorophyll content in fresh foliage of each legume plant was quantified at 90 DAS each for chickpea, pea and lentil and 50 DAS for greengram by the method of Arnon (1949). Briefly, one gram of fresh foliage of each legume was crushed in 40 ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in Buchner funnel having Whatman filter paper No. 1. The residue was grounded three times with acetone and the resulting suspension was filtered again. Contents in mortar-pestle was washed with 80% acetone and filtered. The filtrate was transferred to 100 ml volumetric flask and volume was made upto 100 ml. The absorbance was read at 645 and 663 nm using double beam UV-Visual spectrophotometer (Electronics Corporation of India Limited, India). The total chlorophyll content was calculated as –

$$\textbf{Total chlorophyll} = \frac{[20.2\ (OD_{645}) + 8.02\ (OD_{663})] \times V}{1000 \times W}$$

Where OD_{645} = optical density at 645 nm; OD_{663} = optical density at 663 nm; V = final volume of chlorophyll extract in 80% acetone and W = fresh weight of tissue extracted

3.9.2.4 Nutrient accumulation in legume plants

The total nitrogen content in roots and shoots of chickpea, lentil, pea and greengram were measured at harvest of each legume by the micro-Kjeldahl method of Iswaran and Marwah (1980) while, the P content in the roots and shoots of each legume was measured by the method of Jackson (1967), respectively. Briefly, 50 ml of the sample was taken in the Kjeldahl flask, moistened with 5 ml water, containing 15 ml N/100 ml H_2SO_4 and shaken thoroughly. This was followed by the addition of N $KMnO_4$ in small amount untill pink color appeared. The catalyst mixture (3 g K_2SO_4, 0.3 g $FeSO_4$. 5 H_2O and 0.15 g $CuSO_4$. $5H_2O$) was then added and sample was digested for 30 min. on low flame until the mixture became yellowish green.

3.9.2.4 Seed yield and grain protein

Chickpea, pea, lentil and greengram were finally harvested at 135, 120, 120 and 80 DAS, respectively, and seed yield was measured. The protein content in grains of each legume was estimated by the method of Lowery (1951). For the protein estimation, 500 mg of seeds were soaked in phosphate buffer (pH 7.4) and crushed gently in 5-10 ml phosphate buffer (pH 7.4) (Appendix 31). The extract was centrifuged (4000 rpm) and the supernatant was used for protein analysis. A 0.2 ml aliquot was taken from the sample extract and the volume was made up to one ml in each test tube, followed by addition of 5 ml copper solution (Appendix 32) to each test tube. Each sample was mixed well and allowed to stand for 10 min. and 0.5 ml Folins reagent (Appendix 33) was added to each test tube and incubated at room temperature for 30 min. Absorbance of blue color was read at 660 nm. The protein concentration in the supernatant was determined using a calibration curve of bovine serum albumin (BSA) as a standard.

3.10 Statistical analysis

The experiment was repeated for two consecutive years for all legumes under identical environmental conditions using the same treatments and the resulting data of the measured variables [root length, shoot length, plant dry weight (DW), total chlorophyll content, nodule numbers (Nnod), dry nodule biomass (Mnod), leghaemoglobin (Lb) content, N and P uptake, seed yield (SY) and grain protein (GP)] were pooled together and subjected to analysis of variance. The difference among treatment means was compared by high range statistical domain (HSD) using Tukey test at 5% probability level.

4.1 Microbial diversity in different rhizospheric soils

The rhizospheric soils of mentha, chilli, cabbage, mustard, chickpea, pea, greengram, and lentil, grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were used to determine microbial diversity (Table 14). The viable counts of bacteria, fungi and actinomycetes differed considerably among rhizosphere soils. Generally, the total bacterial populations was highest ($4.28x10^7$ cfu/g soil) while those of actinomycetes was lowest ($1.6x10^4$ cfu/g soil) in all soil samples tested. The order of microbial population in all soil samples was found as: bacteria>fungi>actinomycetes. Among different rhizospheres, the bacterial populations was recorded lowest ($3.42x10^7$ cfu/g soil) in cabbage rhizosphere while in chickpea, pea, greengram and lentil it was $3.62x10^7$, $2.71x10^7$, $4.21x10^7$ and $3.94x10^7$ cfu/g soil, respectively. The rhizospheric soils of mentha, however, showed a considerable increase of 21, 25, and 11% in bacterial populations compared to those recorded for chilli, cabbage, and mustard, respectively. The fungal populations in all the rhizospheric soils ranged from $1.1x10^5$ (lentil) to $1.8x10^5$ (mentha) cfu/g soil. The populations of asymbiotic nitrogen fixer (ANF) for example, *Azotobacter* spp., was also determined in all rhizospheric soil samples. The viable counts of ANF varied noticeably among rhizosphere soils (Table 15). The ANF in all rhizospheric soils ranged between $1.9x10^5$ cfu/g soil (mustard) to $3.2x10^5$cfu/g soil (pea). Moreover, the populations of phosphate solubilizing bacteria (PSB) were greater (mean value $5.24x10^5$ cfu/g soil) in all soil samples than the phosphate solubilizing fungi ($5.20x10^3$ cfu/g) as presented in (Table 14). Similarly, the PSF counts were recorded highest in pea ($6.8x10^3$ cfu/g soil) and lowest in mentha ($3.2x10^3$ cfu/g) rhizospheric soils. While comparing the PSM (including bacteria and fungi) populations in all the rhizosphere soils, the order was: greengram>pea>mentha>chickpea>lentil>chilli>cabbage> mustard.

4.2 Characterization of nitrogen fixing and phosphate solubilizing bacteria

In the present study, a total of 150 symbiotic rhizobia belonging to genera *Mesorhizobium* (N=40), *Rhizobium* (N=70: where 40 were pea rhizobia and 30 were lentil rhizobia) and *Bradyrhizobium* (N=40) were isolated from the nodules of chickpea, pea and lentil, and greengram, respectively, using yeast extract mannitol agar medium. In addition, asymbiotic N_2 fixer such as *Azotobacter* (N=50) and P-solubilizers (N=50) were also isolated from the rhizospheric soils of mentha, chilli, cabbage and mustard. The isolated bacterial cultures showed a variable morphological

and biochemical characteristics (Table 16, 17). Generally, the rhizobial strains were Gram negative while PSB showed a variable Gram reaction. Among the bacterial strains, 38% each of *Mesorhizobium* spp. (chickpea) and *Rhizobium* spp. (pea), 33% each of *Bradyrhizobium* spp. (greengram) and *Rhizobium* spp. (lentil), 40% *Azotobacter* spp. and 36% of PSB were selected for assaying the plant growth promoting activities. Rhizobial strains in general were positive to all the biochemical reactions except methyl red, Voges Proskauer, indole and gelatin hydrolysis test. In contrast, the PSB showed a considerable variation in biochemical properties.

4.3 Functional diversity among plant growth promoting rhizobacteria

In the present study, a total of 70 N-fixers (rhizobia=50 and *Azotobacter*=20) and 30 P-solubilizers were screened for plant growth promoting (PGP) traits such as synthesis of ACC deaminase, phosphate solubilization, IAA, production of siderophores, synthesis of ammonia, hydrogen cyanide and exo-polysaccharides (EPS) and antifungal activity. Based on the PGP activities expressed by the bacterial strains under *in vitro* conditions, the mesorhizobial strains were grouped into four PGP groups (Table 18). All strains of *Mesorhizobium* produced IAA, NH_3 and EPS while 67% strains showed ACC deaminase activity. A total of 47% mesorhizobial strains showed both siderophore and HCN production under *in vitro* conditions. Of these mesorhizobial strains, 13% demonstrated both P-solubilization activity on solid Pikovskaya medium and antifungal activity on PDA plates (Fig.8). The PGP group I included one strains (RG5) which showed eight PGP traits like synthesis of ACC deaminase, P-solubilization, IAA, production of siderophore, synthesis of NH_3, HCN, EPS and antifungal activity followed by group II, which had only one strain (RG4) positive to ACC deaminase, IAA, release of siderophore, secretion of NH_3, HCN, EPS and antifungal activity. In PGP group III, five strains exhibited a positive reaction to ACC deaminase, IAA, production of siderophore, synthesis of NH_3 and EPS, while PGP group IV had three bacterial strains showing positive reaction to ACC deaminase, synthesizing IAA, NH_3, and EPS. The PGP group V, had only one strain (RG6) which showed P- solubilization, IAA, synthesize NH_3 and EPS while PGP group VI included four strains positive for IAA, secreted NH_3, and EPS. All *Rhizobium* strain isolated from pea nodules produced IAA, NH_3 (100%) and EPS where as only 47% strain could synthesize ACC deaminase and HCN. Siderophores, antifungal activity and P-solubilizing activity was shown by 33, 40, and 13% strains, respectively (Fig. 9). Similarly, *Rhizobium* strains isolated from pea nodules were

grouped into three PGP groups (Table 19). The PGP group I included two strains (RP2 and RP6) which showed seven PGP activity like synthesis of ACC deaminase, P-solubilization, IAA, production of siderophore, synthesis of NH_3, HCN and EPS followed by PGP group II, which had three strains positive to ACC deaminase, IAA, production of siderophore, NH_3, HCN, and EPS. Two strains in PGP group III, displayed a positive reaction to ACC deaminase, IAA, synthesis of NH_3, EPS and antifungal activity while group IV included only one strains capable of secreting IAA, NH_3, HCN and EPS. The PGP group V included six strains which synthesized IAA, NH_3 and EPS. Interestingly, all strains of *Bradyrhizobium* were able to synthesize NH_3 and EPS while IAA was produced by 90% strains. Siderophores, HCN, ACC deaminase activity, P-solubilization, and antifungal activity were shown by 50, 50, 20, 10 and 30%, respectively (Fig. 10). *Bradyrhizobium* strains were grouped into six PGP groups wherein each group demonstrated a variable plant growth promoting activities (Table 20). For example, the group I included only one strain (RB6) which showed seven growth promoting traits like, synthesis of ACC deaminase, P-solubilization, IAA, production of siderophore, NH_3, HCN and EPS. The group II had only one strain (RB10) which was found positive to IAA, production of siderophore, synthesis of NH_3, HCN, EPS and antifungal activity. In group III, there were three strains capable of producing IAA, siderophore, NH_3, HCN and EPS while group IV had only one strain which showed ACC deaminase activity, produced IAA, NH_3, and EPS and displayed antifungal activity. The PGP group V included three strains showing the synthesis of IAA, NH_3 and EPS while only one strain belonging to group VI showed positive reaction for NH_3, HCN and EPS. Like other rhizobial cultures, majority of *Rhizobium* strains ($\geq$90%) collected from lentil nodules released NH_3, EPS and IAA. Of these, 50% strains showed both siderophore and cyanogenic activity while ACC deaminase activity, P-solubilization and antifungal activity was demonstrated by 40, 20 and 30%, respectively (Fig. 11).

Rhizobium strains isolated from lentil nodules were placed into eight PGP groups (Table 20). The group I included RV6 and RV8 strains which had ACC deaminase, P-solubilization, IAA, siderophore, NH3, HCN and EPS synthesizing ability while only one strain of group II showed all activities except P-solubilization and antifungal activity. In group III, one strain exhibited a positive reaction to IAA, siderophore, NH_3, EPS and antifungal activity while group IV even-though included only one strain but this strain was found positive for ACC deaminase, IAA, synthesis of NH_3

and EPS. The group V included four strains showed IAA, synthesis of NH_3 and EPS while only one strain of group VI was positive for NH_3, HCN and EPS.

In a similar manner, all strains of asymbiotic N_2 fixer for example *Azotobater* sp. produced IAA, NH_3, and EPS while 55% showed P-solubilizing activity, siderophores production and antifungal activity (55%). The HCN was the least identifiable PGP traits among *Azotobacter* strains (Fig. 12). Like rhizobial strains, the *Azotobacter* strains were also divided into different groups (Table 22). Of these, group I included three strains which showed seven traits (P-solubilization, production of IAA, siderophore, ammonia, HCN, EPS and antifungal activity). The group II in contrast had seven strains which were capable of solubilizing insoluble P, synthesizing IAA, siderophore, NH_3, HCN, EPS and had antifungal activity. Similarly, the members of group III (two strains), IV (five strains) and V (three strains) showed a variable plant growth promoting activity under *in vitro* conditions. Among P-solubilizers, the major activity was the synthesis of IAA, NH_3 and HCN (≥67% strains) while ACC deaminase activity, synthesis of EPS and production of siderophores and antifungal activity was shown by 57, 60, 57 and 53% strains, respectively (Fig. 13). Following these properties, the P-solubilizers were divided into six groups (Table 23) where each group had variable numbers of P-solubilizers and each individual bacterial culture differed greatly in tested plant growth promoting activities. For example, group I included eight strains which showed eight PGP traits such as synthesis of ACC deaminase, P-solubilization, IAA, production of siderophore, synthesis of NH_3, HCN, EPS and antifungal activity.

4.4 Bioassay of plant growth promoting activities

The plant growth promoting substances like ACC deaminase, P-solubilization, IAA, siderophore, HCN, ammonia and exo-polysaccharides synthesized by the PGPR strains were determined both qualitatively and quantitatively under *in vitro* conditions. The antifungal activity of isolated PGPR strains against phytopathogenic fungi namely, *Rhizoctonia* sp., *Alternaria* sp., and *Penicillium* sp. was also determined.

4.4.1 Quantitative analysis of ACC deaminase activity

The isolated rhizobia from nodules and P-solubilizing bacteria from rhizospheric soils were checked for their ability to synthesize ACC deaminase on DF salt medium containing 3 mM ACC instead of $(NH_4)_2SO_4$). Among rhizobia, a total of 11 strains of *Mesorhizobium* (chickpea nodules), seven strains of *Rhizobium* (pea nodules), two

strains of *Bradyrhizobium* (greengram nodules), and four strains of *Rhizobium* (lentil nodules) were positive for ACC deaminase activity. While, 17 P-solubilizers were also found positive for ACC deaminase activity. The ACC deaminase activity was further assessed quantitatively. The analysis of enzyme ACC deaminase was on the basis of production of α-ketobutyrate in µmol by cleavage of ACC. The ACC deaminase activity among *Mesorhizobium* ranged from 113 µmol α ketobutyrate/mg protein/h (RG8) to 258 µmol α ketobutyrate/mg protein/h (RG4) as shown in Table 24. The synthesis of ACC deaminase by *Rhizobium* was variable and differed between 132 µmol α ketobutyrate/mg protein/h (RP10) to 238 µmol α ketobutyrate/mg protein/h (RP2). While among *Bradyrhizobium,* only two strains (RB3 and RB6) were positive for ACC deaminase activity and ACC deaminase activity synthesized by *Rhizobium* isolated from lentil nodules varied between 185 µmol α ketobutyrate/mg protein/h (RV6) to 248 (RV3) µmol α ketobutyrate/mg protein/h (Table 27). The production of ACC deaminase by P-solubilizing bacterial strains also differed significantly. For example, the strain PSE9 produced 227 µmol α ketobutyrate/mg protein/h while strain PSE3 could synthesize 625 µmol α ketobutyrate/mg protein/h (Table 28). Among molecularly characterized P-solubilizers, the *P. putida* strain PSE3 showed maximum ACC deaminase activity (625 µmol α ketobutyrate/mg protein/h) compared to those detected for *Achromobacter* sp. ES1 (163 µmol α ketobutyrate/mg protein/h) and *Pseudoxanthomonas* sp. strain ES5 (578 µmol α ketobutyrate/mg protein/h) (Table 32).

4.4.2 Qualitative and quantitative assay of phosphorus

The plant growth promoting rhizobacteria isolated in this study were further evaluated for phosphate solubilizing (PS) potential, using both solid and liquid Pikovskaya medium supplemented with 5 g/l tri-calcium phosphate (TCP). In the present study, a total of 14% rhizobacterial strains showed PS activity and formed a clear halo around bacterial growth (Plate 2). Among rhizobia, RG5 and RG6 (chickpea), RP2 and RP6 (pea), RB3 and RB6 (greengram) and RV6 and RV9 (lentil) produced halo on solid Pikovskaya which ranged from 6 mm (*Rhizobium* sp. RP6) to 10 mm (*Mesorhizobium* sp. RG5) (Table 24). Of the total *Azotobacter* sp. (N=20), 55% strains demonstrated PS activity and formed 3 (AZ20) to 9 mm halo (AZ19) on solid Pikovskaya medium after five days of incubation. Considering the zone of solubilization and colony diameter of each bacterial strain, the solubilization index (S.I.) was calculated. The SI value rhizobia ranged from 1.4 (*Bradyrhizobium* sp. RB9) to 2.1 (*Bradyrhizobium* sp.

RB6) while for *Azotobacter* spp. it was 1.4 (AZ 20) to 2.6 (AZ 19) and for P-solubilizing bacteria the S.I. varied between 1.5 (*Bacillus* sp. PSE16) to 3.8 (*P. putida* PSE5). The solubilizing efficiency (S.E.) of each P-solubilizer differed from 42 (*Bradyrhizobium* sp. RB9) to 116 (*Bradyrhizobium* sp. RB6) for rhizobia, 50 (*Azotobacter* AZ20) to 150 (*Azotobacter* AZ5) for *Azotobacter* spp. and 50 (*Enterobacter* PSE15) to 333 (*Pseudomonas* PSE3), respectively (Table 29, 30, 31, 32). After evaluating the PS activity of PGPR on solid Pikovskaya medium, the P-solubilization by each bacterial strain was also determined quantitatively. A pattern similar to those observed for solid Pikovskaya medium was recorded for liquid Pikovskaya medium. Generally, the amount of P-solubilized by rhizobia ranged from 45 µg/ml (*Rhizobium* sp. RP6) to 148 µg/ml (*Bradyrhizobium* sp. RB6), 87 µg/ml (*Azotobacter* sp. AZ20) to 215 µg/ml (*Azotobacter* sp. AZ19) among non-symbiotic nitrogen fixers and 111 µg/ml (*Enterobacter* sp. PSE26) to 321 µg/ml (*Achromobacter* PSE28). The P-solubilization by *Achromobacter* strain PSE28 was 189% greater than those observed for lowest P-solubilizing *Enterobacter* strain PSE26. In addition, the solubilization of TCP by different bacterial cultures was coupled with consequent decrease in pH values that ranged between 5.7 (*Rhizobium* sp. RV9) to 6.1 (*Mesorhizobium* sp. RG6) for rhizobia, 5.2 (*Azotobacter* AZ10 and *Azotobacter* AZ19) to 5.9 (*Azotobacter* AZ1) for *Azotobacter* spp. and 4.4 (*Bacillus* PSE21) to 5.8 (*Enterobacter* PSE30). Similarly, the four selected rhizobial strains (RG5, RP2, RB6 and RV9), one *Azotobacter* sp. (AZ19) and eight molecularly characterized P-solubilizers (PSE3, PSE5, PSE9, PSE15, PSE18, PSE19, PSE24 and PSE28) also showed a variable PSA (Table 31 and 32) on both solid and in liquid Pikovskaya medium. The solubilization of TCP by molecularly characterized P-solubilizing bacteria ranged between 163 µg/ml (*Achromobacter* sp. strain PSE9) to 319 (*P. putida* strain PSE3). Of these molecularly characterized P-solubilizers, *P. putida* strain PSE3 showed maximum S.I. (4.1) among other P-solubilizers (Table 24, 25, 26 and 27).

4.4.2.1 Quantitative assay of P-solubilization at different time intervals

Effect of time of incubation on solubilization of P by selected symbiotic N-fixers [(*M. ciceri* strain RG5, *R. leguminosarum* strain RP2, *Bradyrhizobium* strain RB6 and *Rhizobium* strain RV9), asymbiotic N_2-fixers (*Azotobacter* strain AZ19) and P-solubilizers [(*P. putida* strain PSE3 and PSE5, *Achromobacter* strain ES1 and ES6, *Enterobacter* ES2, *B. pumilus* strain ES3 and *Pseudoxanthomonas* strain ES5)] in

Pikvoskaya broth were recorded up to 15 days. Among, rhizobial strains, strain RP2 solubilized maximum amount of P (81 µg/ml) after five days incubation while strain RV9 could solubilize maximum P after 10 (134 µg/ml) and 15 (159 µg/ml) days of growth (Fig. 34). Similarly, *P. putida* strain PSE3, solubilized 319, 412 and 423 µg/ml P after 5, 10 and 15 days of incubation (Fig. 35). While, among P-solubilizer *Achromobacter* strain ES6 solubilized maximally by 321, 411 and 426 µg/ml P after 5, 10 and 15 days of incubation in liquid Pikovskaya medium (Fig. 36).

4.4.3 Bioassay of Indole acetic acid

The production of IAA by the selected bacterial genera namely, *Mesorhizobium ciceri* (N=15), *Rhizobium leguminosarum* (pea, N=15), *Bradyrhizobium* sp (vigna) (N=9), *Rhizobium* spp. (lentil, N=9), *Azotobacter* sp. (N=20) and P-solubilizers (N=30) was assayed in LB broth treated with (100 µg/ml) or without (0 µg/ml) tryptophan. The *M. ciceri* exhibited a substantial production of IAA after three days incubation. Moreover, a wide range of variability in the secreted amount of IAA was observed among rhizobial isolates. The amount of IAA synthesized by mesorhizobial strains varied between 14 (RG14) to 29 µg /ml (RG10) in LB broth without tryptophan and 32 (RG14) to 75 µg /ml (RG4) in LB broth supplemented with 100 µg/ml tryptophan (Table 24). While comparing the IAA synthesis by mesorhizobial strains at 100 µg tryptophan/ml, the strain RG4 showed a maximum increase of 134% in IAA production over strain RG14. Among the pea specific *Rhizobium* isolates, strain RP9 produced a detectable amount of 32 (0) and 73 µg/ml IAA (100 µg tryptophan/ml). This was followed by strain RP8 which produced 28 and 75 µg IAA/ml at 0 and 100 µg/ml tryptoptophan, respectively. The amount of IAA synthesized by rhizobial strains varied between 13 (RP15) to 32 µg /ml (RP9) at 0µg/ml tryptophan and 41 (RP3) to 75 µg /ml (RP8) at 100 µg/ml tryptophan, respectively. The percent increase in IAA synthesized by RP9 over other rhizobial strains ranged between 30 (RP12) to 146 (RP15) (Table 25). *Bradyrhizobium* strains also produced a significant amount of IAA, maximum being 95 µg/ml IAA by the strain RB4 followed by 85 µg/ml IAA at 100 µg/ml tryptophan, respectively. The increase in IAA synthesized by RB4 maximum over other rhizobial strains (Table 25). Similarly, the *Rhizobium* strains isolated from lentil nodules showed a variable amount of IAA (Table 26). For instance, strain RV6 and RV3 produced a 92 and 89 µg/ml IAA at 100 µg/ml tryptophan, respectively. The increase in IAA synthesized by RV6 over other

rhizobial strains ranged between 3 (RV3) to 57% (RV2) when LB broth was treated with 100 µg/ml tryptoptophan (Table 27).

The IAA production by *Azotobacter* sp. (N=20) was also assayed in a manner similar to those detected for other bacterial genera (Table 28). Of these, *Azotobacter* sp. AZ19 and AZ4 was most effective and produced 96 and 89 µg/ml IAA at 100 µg/ml tryptophan, respectively. The increase in IAA synthesized by AZ19 over other *Azotobacter* strains ranged between 10 (AZ10) to 121% (AZ14) and 5 (AZ20) to 81% (AZ3) at 0 and 100 µg/ml tryptoptophan, respectively. Similarly, the IAA production by PS bacteria (N=30) differed with both variation in bacterial strains and tryptophan concentrations applied (Table 29, 30, 31, 32). Among P-solubilizers, PSE25 maximally produced IAA (62 µg/ml) which was followed by PSE24 (62 µg/ml IAA) when grown in LB broth treated with 100 µg/ml tryptoptophan. The increase in IAA synthesized by *Enterobacter* PSE25 while growing in LB broth treated with 100 µg/ml tryptoptophan over other P-solubilizers strains ranged between 8 (PSE21) to 172% (PSE3). Generally, the synthesis of IAA by all molecularly characterized P-solubilizers was greater when grown in medium treated with tryptophan than those recorded for untreated medium. For example, the IAA production by molecularly identified bacterial cultures ranged from 14 (*Achromobacter* sp. strain ES1) to 27 µg/ml IAA (*Pseudoxanthomonas* sp. strain ES5) and 34 (*Achromobacter* sp. strain ES1) to 62 µg/ml IAA (*Pseudoxanthomonas* sp. strain ES5) in LB broth treated with 0 and 100 µg/ml tryptoptophan, respectively. Both at 0 and 100 µg/ml tryptoptophan, the synthesis of IAA by P-solubilizers followed the order: *Pseudoxanthomonas* sp. strain ES5> *Stenotrophomonas* sp. strain ES4> *Enterobacter* sp. ES2> *Bacillus pumilus* ES3=*P. putida* strain PSE3 >*Achromobacter* sp. strain ES1.

4.4.3.1 Effect of Tryptophan concentration and time dependent production of IAA

The effect of tryptophan concentration and incubation intervals on production of IAA by selected [symbiotic N_2-fixers (*M. ciceri* strain RG5, *R. leguminosarum* strain RP2, *Bradyrhizobium* strain RB6 and *Rhizobium* strain RV9)] and [asymbiotic N_2-fixers (*Azotobacter* strain AZ19)] and P-solubilizers (*P. putida* strain PSE3 and PSE5, *Achromobacter* strain ES1 and ES6, *Enterobacter* ES2, *B. pumilus* strain ES3 and *Pseudoxanthomonas* strain ES5) was assayed. All bacterial strains were inoculated in LB broth containing 0, 50, 100, 200, 400 and 500 µg/ml tryptophan and the IAA was assayed at 4,8,12 and 16 days time intervals. Impact of incubation intervals on

production of IAA by four symbiotic N-fixers was recorded up to 16 days at an interval of 4 days. A detectable amount of IAA was produced by all strains after 4 days growth; level of IAA however, varied with time and concentration among RG5 (Fig. 20), strain RP2 (Fig. 21) strain RB6 (Fig. 22) and strain RV9 (Fig. 23). Generally, the production of IAA was increased with increasing concentration of tryptophan but there were little difference in the synthesis of IAA between the incubation intervals among rhizobial strains. For example, *Rhizobium* strain RV9 produced IAA 65, 74, 90, 115 and 123 µg/ml at 50, 100, 200, 400 and 500 µg/ml tryptophan concentration, respectively after four days of incubation. While, strain RV9 synthesized IAA 123, 125, 128 and 129 µg/ml at 4, 8, 12 and 16 days, respectively 500 µg/ml tryptophan concentration (Fig. 25). Further, *Azotobacter* strain AZ19 also synthesized the IAA maximally at 500 µg/ml tryptophan and showed an increase of 242% over IAA produced at 50 µg/ml tryptophan after 4 days of incubation (Fig. 24). Likewise, among P solubilizers, *P. putida* strain PSE3 (Fig. 25) and PSE5 (Fig. 26) also displayed enhanced synthesis of IAA at the highest test rates of tryptophan after 4 days of incubation while, *B. pumilus* strain ES3 produced maximum IAA at 500 µg/ml tryptophan after 16 days of incubation (Fig. 29).

4.4.4 Bioassay of siderophores

In the present investigation, the production of siderophores was determined both qualitatively and quantitatively using CAS agar and ethyl acetate extraction method. On CAS agar plates, a total of 47% of the *Mesorhizobium* strains produced siderophore (Fig. 8). The siderophore was detected by the formation of a visible orange yellow halo on CAS agar plates after five days of incubation whose size varied between 10 (*Mesorhizobium* RG1) to 12 mm (*Mesorhizobium* RG3 and RG8). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain RG8 yielded 16 and 33 µg/ml of 2,3-dihydroxy benzoic acid (DHBA) and salicylate (SA), strain RG7 produced 15 and 34 µg/ml of DHBA and SA, strain RG5 yielded 13 and 28 µg/ml of DHBA and SA, and strain RG4 produced 15 and 29 µg/ml of DHBA and SA, respectively. Comparing the siderophore production among the siderophore producing mesorhizobial strains, strain RG7 substantially increased the DHBA and SA by 25% and 32%, respectively, over the lowest siderophore producing mesorhizobial strain RG3 (Table 24). Similarly, 33% of the *Rhizobium* strains isolated from pea nodules showed a positive siderophore activity (Fig. 9). Generally, the size of orange yellow colored zone appearing on CAS plates after five days of incubation

was greater than 10 mm. Additionally, the rhizobial strains produced 12 and 24 (strain RP2), 15 and 19 (RP3), 16 and 28 (RP6), 14 and 27 (RP8) and, 13 and 31 (RP10) µg/ml DHBA and SA, respectively. Among the siderophore positive rhizobial strains, strain RP6 maximally enhanced the DHBA by 25% relative to the poorly DHBA secreting strain RP2 while strain RP10 showed maximum increase in SA (63%) compared to the lowest SA producing strain RP3 (Table 25). Strains RB1, RB2, RB6, RB7 and RB10 of *Bradyrhizobium* species showed 12, 11, 12, 10 and 11 mm orange yellow colored zone, respectively, on CAS agar plates after five days of incubation and produced 15 and 31 (strain RB1), 16 and 35 (RB2), 14 and 25 (RB6), 15 and 31 (RB7), and 14 and 26 (RB10) µg/ml DHBA and SA respectively. Among siderophore positive bradyrhizobial strains, strain RB3 considerably enhanced DHBA by 14% while it increased the SA by 40% in comparison to the lowest siderophore synthesizing strain RB6 (Table 26). In a similar manner, the siderophores assayed both qualitatively and quantitatively for the *Rhizobium* species isolated from lentil nodules (Table 27) and *Azotobacter* species isolated from various rhizosphere soils, varied greatly (Table 28). In other experiments, a total of 57% of the P-solubilizing bacterial strains produced siderophore on CAS agar plates and the halo size ranged between 10 (strain *Enterobacter* PSE10) to 13 mm (*Pseudomonas* PSE6, *Enterobacter* PSE15, *Pseudoxanthomonas* sp. PSE19, *Enterobacter* sp. PSE26 and *Enterobacter* sp PSE29). Quantitatively, the amount of DHBA and SA among P-solubilizers differed between 14 (*P. putida* PSE5 and *B. pumilus* PSE18) to 21 (*Bacillus* sp. PSE22 and *Achromobacter* PSE28) and 28 (PSE5) to 38 µg/ml (*Bacillus* sp. PSE22), respectively. Strain PSE22 among all P-solubilizers dramatically increased the DHBA and SA by 90 and 35%, respectively over the lowest siderophore producing P-solubilizing strain PSE5 (Table 29, 30, 31 and 32).

4.4.5 Bioassay of exo-polysaccharides

The culture supernatant was used to determine the exo-polysaccharides (EPS) secreted by the PGPR strains. Generally, the amount of EPS released by rhizobacteria varied considerably among bacterial species including both N_2 fixers and P-solubilizers after five days incubation. Among symbiotic nitrogen fixers, the mesorhizobial strains especially strain RG4 had a maximum amount (32 µg/ml) of EPS and exhibited a considerable increase in EPS compared to the lowest EPS (19 µg/ml) producing bacterial strain RG9 (Table 24). Among *Rhizobium* strains isolated from pea nodules, even-though all strains were able to secrete EPS, the strain RP6

was found superior and produced a maximum amount (35 µg/ml) of EPS. Strain RP13 in contrast showed a very poor synthesis of EPS (15 µg/ml) as presented in Table 25. *Bradyrhizobium* strains also produced a significant amount of EPS, the maximum being 26 µg/ml EPS observed for the strain RB5 and it was considerable increase (85%) compared to lowest EPS (14 µg/ml) producing strain RB9 (Table 26). *Rhizobium* strains specific to lentil plants like other rhizobial strains also showed a variable amount of EPS (Table 27). For example, strain RV1 synthesized highest amount (31µg/ml) of EPS which was followed by strain RV6 (28 µg/ml). The EPS production by *Azotobacter* sp. ranged between 19 (AZ12 and AZ19) to 45 µg/ml (AZ3). Among all *Azotobacter* species, the EPS released by strain AZ3 was 126% more than those recorded for strain AZ19 (Table 28). The EPS production by P-solubilizing bacteria ranged between 13 (PSE20) to 25 µg/ml (PSE3 and PSE30) as shown in Table 28. The EPS synthesized by the strain PSE3 was and 92% higher compared to the lowest EPS producing strain PSE3 among all P-solubilizers (29, 30. 31 and 32).

4.4.6 *In vitro* assay of ammonia and HCN

The plant growth promoting rhizobacterial strains were tested further for the synthesis of ammonia and cyanogenic compounds (e.g., hydrogen cyanide) use peptone water and HCN induction medium, respectively. Interestingly, all N_2-fixers (rhizobia and *Azotobacter*) and P-solubilizers showed a positive reaction for ammonia (Fig. 8, 9, 10, 11, 12, and 13). In contrast, among symbiotic N_2-fixers, 47, 47, 50 and 50% strain of *Mesorhizobium*, *Rhizobium* (pea), *Bradyrhizobium* and *Rhizobium* (lentil), respectively showed cyanogenic activity while, 50% of *Azotobacter* strains were found positive to HCN. Furthermore, a total of 67% P-solubilizers showed cyanogenic activity (Fig. 8, 9, 10, 11, 12, and 13).

4.4.7 Antifungal activity of N_2-fixers and P-solubilizers

Antifungal activity of N_2-fixers (N=70) and P-solubilizers (N=30) was assessed on PDA which differed considerably against three phytopathogens, namely, *Rhizoctonia* sp., *Penicillium* sp. and *Alternaria* sp. (Plate 4). Among, rhizobial group, 13, 20, 20 and 30% strains of *Mesorhizobium*, *Rhizobium* (pea), *Bradyrhizobium* and *Rhizobium* (lentil) respectively, inhibited the growth of the tested phytopathogens while 55% strains of *Azotobacter* sp. showed a visible antifungal activity on pathogens inoculated PDA plates. Similarly, 53% strains of P-solubilizers isolated from rhizospheric soils

had the strong inhibitory impact on *Rhizoctonia* sp., *Penicillium* sp. and *Alternaria* sp. Among, *Mesorhizobium,* strain RG4 showed a 24, 21 and 23 mm size zone of inhibition on PDA while RG5 showed 21, 19 and 23 mm inhibition zone against *Rhizoctonia* sp., *Penicillium* sp. and *Alternaria* sp., respectively (Table 33). The *Rhizobium* strain RP5 isolated from pea produced the largest zone of inhibition against *Penicillium* sp. (28 mm) which was followed by *Alternaria* sp. (27 mm) and *Rhizoctonia* sp. While, RP6 showed a 25, 24 and 25 mm size zone of growth inhibition on PDA and RP8 showed 32, 29 and 30 mm growth inhibition zone against sp., *Penicillium* sp. and *Alternaria* sp., respectively. Among asymbiotic N_2-fixer *Azotobacter* sp., strain AZ13 exhibited the largest inhibitory effect against *Penicillium* sp. (32 mm), *Rhizoctonia* sp., (28 mm) and *Alternaria* sp. (28 mm) followed by strain AZ17 which maximally inhibited the growth of *Penicillium* sp. (29 mm), *Rhizoctonia* sp. (25 mm) and *Alternaria* sp. (24 mm). Similarly, strain AZ11 of *Azotobacter* also showed greater ability to inhibit the growth of fungal pathogen and produced zone of inhibition sizes of 29, 26 and 24 mm against *Penicillium* sp., *Rhizoctonia* sp. and *Alternaria* sp., respectively. The AZ13 strain demonstrated maximum inhibition against phytopathogenic fungi and showed a profound increase in zone of inhibition over AZ6 against *Penicillium* sp., (128%), *Rhizoctonia* sp. (86%) and *Alternaria* sp. (100%). Similarly, P-solubilizers showed variable antifungal activity against tested phytopathogenic fungi. Among, P-solubilizers, PSE24 showed maximum zone of inhibition against *Penicillium* sp. (32 mm), *Rhizoctonia* sp. (28 mm) and *Alternaria* sp. (29 mm) followed by strain PSE28 which inhibited the growth of *Penicillium* sp. (29 mm), *Rhizoctonia* sp. (27 mm) and *Alternaria* sp. (25 mm) considerably (Table 34).

4.5 Antibiotic sensitivity of bacterial isolates

The sensitivity/resistance profile of N_2-fixers and P-solubilizers was determined using disc diffusion method (Table 35). All rhizobia in general were more sensitive to tested antibiotics. Of these, *Rhizobium* strain RV9 was most sensitive (64.7%) to all antibiotics while *Rhizobium* strain RP2 showed resistance to about 58.8% antibiotics. Similarly, *Azotobacter* strains showed a variable sensisitivity/resistance toward different antibiotics. Among P-solubilizers, *Pseudoxanthomonas* strain PSE19 *Stenotrophomonas* showed maximum (70.6% each) resistance to test antibiotics.

4.6 Identification of selected PGPR strains by 16S rRNA sequencing

On the basis of cultural, morphological and biochemical characteristics and comparing such properties with those given in Bergey's Manual of Determinative Bacteriology, the plant growth promoting rhizobacteria isolated from host specific nodules of legume plants were tentatively grouped as *Mesorhizobium* spp. (chickpea), *Rhizobium* spp. (pea), *Rhizobium* spp. (lentil) and *Bradyrhizobium* spp. (greengram). Non symbiotic N-fixer was also identified as *Azotobacter* on the basis of cultural, morphological and biochemical characteristics and pigment (melanin) production. While, other PGPR isolated in this study belonged to genera, *Pseudomonas*, *Bacillus*, *Achromobacter*, *Enterobacter*, *Pseudoxanthomonas* and *Stenotrophomonas*. Moreover, eight isolates namely, PSE3, PSE5, ES1, ES2, ES3, ES4, ES5 and ES6 showing highest degree of TCP solubilization and greatest ACC deaminase activity were selected for genetic characterization to species level using 16S rRNA gene analysis (Table 34). The molecular characterization was done commercially by Macrogen Inc., Seoul, South Korea. All nucleotide sequences were examined by nucleotide sequence based BLASTn analysis from NCBI online server to confirm the maximum identity with other bacteria. The 16S rRNA partial genome sequences were then submitted to NCBI nucleotide based GenBank and accession number was obtained for each sequence. The bacterial strains were identified as *Pseudomonas putida* strain PSE3 and PSE5 (Gene Bank accession number HM236047 and HM236047), *Achromobacter* strain ES1 and ES6 (Gene Bank accession number JX483710 and JX 965905), *Enterobacter* strain ES2 (Gene Bank accession number JX 965901),) *Bacillus pumilus* strain ES3 (Gene Bank accession number JX 965902), *Pseudoxanthomonas* strain ES4 (Gene Bank accession number JX 965903) and *Stenotrophomonas* strain ES5 (Gene Bank accession number JX 965904), respectively, as depicted in Table 36.

4.7 Phylogenetic t ·ee of molecularly characterized bacterial

Phylogenetic tree of eight P-solubilizer strains was constructed with the help of MEGA 4.1 software and BLASTn analysis of 16S rRNA gene sequences. A comparative analysis of 16S rRNA genes of bacterial strains was done by constructing maximum parsimonious phylogenetic consensus tree with reference sequences from the NCBI GenBank data base and the phylogenetic tree was constructed for each of *P. putida* strain PSE3 and PSE5 (Fig. 14), *Achromobacter* strain ES1 and ES6 (Fig. 15),

Enterobacter strain ES2 (Fig. 16), *B. pumilus* strain ES3 (Fig. 17), *Pseudoxanthomonas* strain ES4 (Fig 18) and *Stenotrophomonas* strain ES5 (Fig. 19).

Pot and field trails

Chickpea

4.8 Length of plant organs

4.8.1.1 Root

In this experiment, the inoculated and uninoculated chickpea plants were grown in sandy clay loam soils treated with or without basal dose of synthetic chemical fertilizers and the length of plant organs (root and shoot) were measured at different intervals. The length of roots was increased by 15 (24.3 cm) and 21% (25.5 cm) when plants were grown in soil treated with 30 kg urea/ha and 80 kg DAP/ha, respectively in pot trials after 90 DAS (Table 38) compared to uninoculated and untreated control plants (21.1 cm). In contrast, there was a marginal increase in root length of chickpea plants when grown under field trials. The root length was however, substantially increased by 46, 43 and 38% after 90 days of growth when *P. putida* strain PSE3, *B. pumilus* strain ES3 and *Azotobacter* strain AZ19 bacterized seeds, respectively, were grown in pot soils treated with 30 kg urea/ha. In field trials, the *P. putida*, *B. pumilus* and *Azotobacter* inoculated chickpea plants grown in soils treated with 30 kg/ha had 33, 31 and 29% more root growth, respectively, compared to control at 90 DAS. In a similar experiment, when 80 kg/ha DAP was applied together with *M. ciceri* in field trials the root length was increased by 37 (31.2 cm) and 35% (32.3 cm) after 90 and 135 days of growth, respectively over uninoculated and untreated control plants. The coinoculation effect of P-solubilizer, *P. putida*, *B. pumilus* and *Azotobacter* with symbiotic N-fixer, *M. ciceri* on root length of chickpea plant observed at 90 and 135 DAS in both pot and field trials was variable and it was significantly ($P \leq 0.05$) increased by 59, 58 and 53% in pot trials after 90 DAS as compare to control. While, 45, 50 and 37% increase in root length was recorded when plant was coinoculated with *P. putida*, *B. pumilus* and *Azotobacter* with *M. ciceri,* respectively, 90 days after sowing in field trials (Table 38, 42 and 46). A similar increase in the length of plant root was observed at 135 DAS when plants were grown in soils treated with or without nitrogenous or phosphatic fertilizers and bio-primed plants. However, a substantive increase in plant organs was recorded at 135 DAS compared to 90 DAS.

4.8.1.2 Shoot

The shoot length of inoculated or uninoculated plants grown in pot/field soils receiving urea and DAP applied either alone or as mixture was recorded at 90 and 135 DAS. The shoot length was increased by 21, 29 and 54% when plants were grown in pot soils supplemented with 30 kg/ha urea, 80 kg/ha DAP and mixture of 30 kg/ha urea with 80 kg/ha DAP, respectively, compared to control (Table 38). At 30 kg/ha urea, 80 kg/ha DAP and mixture of 30 kg/ha urea with 80 kg/ha DAP, applied in field trials, this increase was found as 17, 24 and 43%, respectively, at 90 DAS in comparison to control. When *P. putida*, *B. pumilus* and *Azotobacter* seeds were sown and grown in pot soils amended with 30 kg urea/ha urea the shoot length was found to increase by 42 (35.6 cm) 33 (34.2 cm) and 32% (33.3 cm) respectively, at 90 DAS compared to control plants (Table 38, 42 and 46). In contrast, at 80 kg/ha DAP, applied to field soils, the shoot length of the *M. ciceri* inoculated chickpea increased by 47% as compared to control plants 90 days after sowing. The shoot length was maximally increased by 59, 61 and 52% respectively as compare to control when plants was coinoculated with P-solubilizers *P. putida*, *B. pumilus* and *Azotobacter* with N-fixer *M. ciceri* at 90 DAS in pot trials. In general, the application of phosphatic fertilizer (DAP) used either alone or as mixture with *M. ciceri*, had more pronounced impact on root and shoots growth of chickpea plants grown either in pot or field environment. While comparing the effect of fertilizers (nitrogenous and phosphatic), inocula or culture with fertilizers, the combined application of urea and DAP was found superior over all treatments except the dual inoculation of *M. ciceri* with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter*.

4.8.2 Dry matter accumulation

4.8.2.1 Root

Dry matter accumulated within roots of chickpea growing plants following bio-inoculants and fertilizer application measured at different stages of growth was variable when grown in pot and field conditions. The application of N-fertilizer (urea) @ 30 kg/ha and P-fertilizer (DAP) @ 80 kg/ha and mixture of both fertilizers (30 kg/ha urea+80 kg/ha DAP) increased the dry root biomass from 0.93 g/plant (control) to 1.23g/plant (32%), 1.25 g/plant (34%), and 1.41 g/plant (52%), respectively in pot trials and from 1.12g/plant (control) to 1.34 g/plant (20%), 1.38 g/plant (23%) and 1.45 g/plant (31%) in field trials at 90 DAS (Table 39). In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida*

(ii) *B. pumilus* and (iii) *Azotobacter* enhanced the root dry biomass by 50, 49 and 46%, respectively, in pot trails at 90 DAS compared to un-inoculated and untreated control plants (39, 43 and 47). The sole application of *P. putida, B. pumilus*, and *Azotobacter* and *M. ciceri* augmented the dry matter accumulation in roots by 40, 43, 38 and 39%, respectively at 90 DAS compared to control plants. The dry root biomass was increased further by 29, 31, and 27% in pot trials while in field trials this increase was 47, 35 and 27%, at 135 DAS following *M. ciceri* inoculation with *P. putida*, *B. pumilus* and *Azotobacter*, respectively. The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials.

4.8.2.2 Shoot

The dry matter accumulation in shoots of chickpea plants growing in soils (pot and field) treated with urea, DAP or both and inoculated or coinoculated with *P. putida*, *B. pumilus* and *Azotobacter* with *M. ciceri* differed profoundly both at 90 and 135 DAS. The single application of P-solubilizers *P. putida*, *B. pumilus*, *Azotobacter* and symbiotic N_2-fixer *M. ciceri* increased the dry shoot biomass in chickpea plants by 26, 19, 16 and 18% (in pot trials) and by 17, 19, 16 and 23%, respectively (in field conditions) at 90 DAS which was increased even further at 135 DAS compared to un-inoculated and untreated control plants (Table 39). Among the synthetic fertilizers, the combination of both urea and DAP increased the dry matter accumulation in shoots maximally by 24 and 35% compared to control at 90 and 135 DAS, respectively in pot trials. The shoot dry biomass of chickpea plants raised under field soils was increased considerably by 21, 23 and 34% at harvest (135 DAS) when plants was grown only with 30 kg urea/ha, 80 kg DAP/ha and 30 kg urea/ha with 80 kg DAP/ha, respectively. In comparison, the composite application of fertilizers and microbial inocula increased the shoot dry biomass by 54%, each with urea with *P. putida* and DAP with *M. ciceri* in pot trails and 74 (urea with *P. putida*) and 47% (DAP with *M. ciceri*) in field conditions, respectively, at 90 DAS compared to un-inoculated and untreated control plants. The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field soils.

4.8.2.3 Total dry biomass accumulation

Total dry matter accumulated in chickpea plants following bio-inoculants and fertilizer application measured at different stages of growth was variable when grown

in pot and field conditions. The application of N-fertilizer (urea) @ 30 kg/ha and P-fertilizer (DAP) @ 80 kg/ha and mixture of both fertilizers (30 kg/ha urea+80 kg/ha DAP) increased the total dry biomass from 3.04 g/plant (control) to 3.58 g/plant (17%), 0.3.66 g/plant (20%), and 4.02 g/plant (32%), respectively in pot trials and from 3.32 g/plant (control) to 3.79 g/plant (14%), 3.89 g/plant (17%) and 4.57 g/plant (38%) in field trials at 90 DAS. Similarly, at 135 DAS, the total dry matter accumulation in chickpea plants grown both in pots and field soils differed among treatments (Fig. 36). Furthermore, the sole application of *P. putida, B. pumilus*, *Azotobacter* and *M. ciceri* augmented the dry matter accumulation in plants by 24, 27, 23 and 24%, respectively at 90 DAS compared to control plants. At 135 DAS, the increase in dry matter accumulation in roots was 26 (*P. putida*), 27 (*B. pumilus*), 23 (*Azotobacter*) and 24% (*M. ciceri*) in pot experiments. In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the total dry biomass by 29, 30 and 32%, respectively, in pot trails at 90 DAS while it was 31, 32 and 30% at 135 DAS compared to un-inoculated and untreated control plants (Fig. 36, 38 and 40). Total dry biomass was increased further by 33, 37, and 32% in pot trials while in field trials this increase was 31, 32 and 30%, at 90 DAS following *M. ciceri* inoculation with *P. putida* (Fig. 37), [*B. pumilus* (Fig. 39) and *Azotobacter*, respectively (Fig. 41). The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials.

4.8.3 Photosynthetic pigments and symbiotic attributes

In the presence of 80 kg DAP/ha, *M. ciceri* enhanced the chlorophyll content from 0.1.54 mg/g (control) to 1.77 mg/g in pot grown plants while in field, it increased the chlorophyll content from 1.45 mg/g (control) to 1.89 mg/g (Table 38). The chlorophyll content was increased by 12, 13, 11 and 10% in fresh foliage when chickpea plant was inoculated with sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *M. ciceri*, respectively, in pot trials. While 12, 11 and 18% enhancement in chlorophyll content was recorded when plant was gown in pot soils receiving 30 kg/ha urea, 80 kg/ha DAP and mixture of urea with DAP respectively. The dual application of P-solubilizers (*P. putida*, *B. pumilus*, *Azotobacter*) with N-fixer (*M. ciceri*) in general, had maximum positive impact on chlorophyll formation of chickpea plants raised both in pot and field trials. The co-cultures of *M. ciceri* with

P. putida, *B. pumilus* and *Azotobacter* maximally increased the chlorophyll content in fresh foliage by 17.5, 18.1 and 16% (Table 40, 44, 48) respectively, as compared to control plants grown in pots while in field, it was 34, 35 and 31%, respectively.

4.8.4 Nodulation and leghaemoglobin content

The formation and distribution of nodules onto the root systems of inoculated or uninoculated chickpea plants measured at podfill stage of growth was variable when grown in pot and field conditions. Microbial cultures in general facilitated the formation of nodules on the root systems of chickpea plants compared to urea or DAP. As an example, the single application of *M. ciceri* was found superior over synthetic fertilizers and maximally increased the nodule numbers, nodule dry biomass , and leghaemoglobin (Lb) contents of field grown chickpea plants by 74, 36, and 56% over plants grown in field soils treated only with 30 kg urea/ha urea (Table 40). Generally, the sole or combined application of *M. ciceri* as host specific inoculant showed a prolific impact on symbiotic attributes of chickpea plants compared to other single or simultaneous application of bacterial cultures. For example, the nodule numbers, nodule dry biomass and Lb contents in fresh nodules were increased significantly ($P \leq 0.05$) by 126, 137 and 87% in pot trails while this increase in field trials was 100, 137 and 112%, respectively due to inoculation with *M. ciceri* alone over uninoculated but untreated control plants (Table 40). In contrast, the sole application of *P. putida* enhanced the the nodule numbers, nodule dry biomass and Lb by 96, 118 and 60% in greenhouse conditions and 52, 113 and 83%, in natural conditions, respectively, compared to control. Similarly, *B. pumilus* increased the the nodule numbers, nodule dry biomass and Lb content by 109, 129 and 60% in pot conditions and 59, 103 and 94%, in field soils, respectively, over control (Table 44). While comparing the impact of fertilizers on measured parameters, the 30 kg urea/ha, 80 kg DAP/ha and mixture of both fertilizers increased the nodule numbers by 15, 19 and 67%, respectively while the Lb content was improved by 35, 47 and 94%, respectively compared to control plants grown in field trials. Among the two fertilizers, DAP in general, maximally induced nodule formation on chickpea plants when used either alone or in combination with microbial cultures. The co-inoculation of N_2-fixer *M. ciceri* with- (i) P-solubilizers, *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* increased the the nodule numbers, nodule dry biomass and Lb content by- (i) 122, 137 and 107% (Table 40) (ii) 122, 137 and 106% (Table 44) and (iii) 121, 137 and 106%, (Table 48) compared to plants raised in pot soils. The nodule biomass of other inoculated and uninoculated plants followed a trend similar to those observed for nodule formations.

4.8.5 Concentration and uptake of N and P

The effect of microbial inoculations/fertilization on the concentration and uptake of N and P by chickpea plants differed among treatments (Table 41, 45, 49). Single inoculation of *M. ciceri*, *P. putida* and *B. pumilus*, and *Azotobacter* significantly increased the N contents by 93 and 42% (root:shoot), 87 and 31% (root:shoot), 80 and 23% (root:shoot) and 73 and 23% (root : shoot) at 135 DAS compared to the control plants grown in pots. Similarly, under field trials, the *M. ciceri*, *P. putida*, *B. pumilus*, and *Azotobacter* significantly enhanced the N contents in roots and shoots of chickpea plants by 111 and 35%, 78 and 26%, 67 and 19% and 19 and 78%, respectively. The P concentration in roots and shoots of chickpea plants grown in pots following sole application of *M. ciceri*, *P. putida* and *B. pumilus*, and *Azotobacter* significantly by 63 and 63%, 69 and 67%, 75 and 74% and 56 and 55%, respectively. Similarly, under field trials, the *M. ciceri*, *P. putida*, *B. pumilus*, and *Azotobacter* significantly enhanced the P contents in roots and shoots of chickpea plants by 61 and 53%, 98 and 52%, 94 and 56% and 78 and 48%, respectively. While comparing the impact of single microbial cultures on N and P contents in roots and shoots of chickpea plants grown both in pots and field soils, it was observed that *P. putida* had the largest stimulatory effect on N and P concentration (Table 41). In a similar way, when effect of single application of microbial cultures was compared with those of urea and DAP used alone, it was found that microbial cultures in general had better effect on N and P concentration in both roots and shoots of pot and field grown chickpea plants. The co-inoculation of [*M. ciceri* and *B. pumilus*] maximally increased the N concentration in roots and shoots by 187 and 97% (pots), 150, 71% (field) while P content was enhanced by 144 and 88% (pot trails) and 167 and 96% (field conditions) above the control plants grown both in pots and field. Similarly, the combined application of *M. ciceri* and *P. putida* enhanced the N and P contents in roots and shoots by 1.8, 0.85, 1.4, and 1.4 fold (pot trails) and 1.5, 0.7, 1.7 and 0.9% (field conditions) above the un-ionoculated and untreated control. Among fertilizers, DAP had maximum positive effect and increased the root N (24 mg/g), root P (0.24mg/g) and shoot P (0.29 mg/g) by 12, 50 and 50%, respectively whereas the shoot N (31 mg/g) was maximally increased by 60% when chickpea plants was grown in soil treated with urea as compared to control plants grown in pot trials. *M. ciceri* inoculated plants grown in soil treated with DAP increased the N contents in roots and shoots and P contents in

roots and shoots by 153, 69, 112 and 111% in pot trials and 116, 48, 127 and 74% in field conditions, respectively over non inoculated and non- treated control plants (Table 41). Similarly, the mixture of both urea (30 kg/ha) and DAP (80 kg/ha) showed maximum increase in the measured parameters compared to other single treatment of urea or DAP or control plants in both pot and field trials (Table 41). Of the two fertilizers, DAP in general, had maximum positive effect on the measured parameters of both inoculated and un-inoculated chickpea plants.

4.8.6 Seed yield and seed protein

A single inoculation with *M. ciceri*, *P. putida*, *B. pumilus*, and *Azotobacter* significantly ($P \leq 0.05$) increased the seed yield by 54, 57, 64 and 50% relative to the control (Tab 41, 45, 49). The dual combination of [*P. putida* strain PSE3 and *M. ciceri* strain RG5] increased the grain yield maximally by 111% and 138% respectively in pot and field trials which was followed by *B. pumilus* and *M. ciceri* inoculated chickpea plants grown in pots (121%) and fields (111%) compared to control plants. Mixed application of recommended dose of urea and DAP increased the seed yield by 82% and 112% in pot (5.1g/plant) and field (5.5 g/plant) conditions, respectively over control. Moreover, in the presence of 30 kg urea/ha, *P. putida* had the maximum positive effect on seed formation in chickpea and increased it significantly ($P \leq 0.05$) by 82% while *M. ciceri* in the presence of 80 kg DAP/ha augmented the seed yield by 89% compared to untreated and uninoculated plants grown in pots. Under field trials, *M. ciceri* in the presence of 80 kg DAP/ha gave maximum seed yield (112% increases) compared to control. Among all treatments including microbial cultures and fertilizers, the co-cultures of [*M. ciceri* and *B. pumilus*] had the maximum impact both in pots and field trials and resulted in 175% increase in seed yield over control plants grown in pots. The protein content in chickpea seeds even-though did not differ significantly among treatments yet it was greater in inoculated plants compared to control ones (Table 41). The two way ANOVA in general revealed that the individual effect of inoculants (df=3), further application of fertilizer (df=1) and interaction between inoculation and fertilizer (df=3) was significant ($P \leq 0.05$) for all measured parameters.

4.9 Length of plant organs

4.9.1.1 Root

The inoculated and uninoculated peas had variable biological properties when grown in sandy clay loam soils treated with or without synthetic chemical fertilizers (Table 50). The growth of plant organs also varied from organisms to organisms and stages of plant development. The length of the underground parts (roots) of pea was increased by 23 (19.3 cm) and 40% (22.1 cm) when plants were grown in soil treated with 20 kg urea/ha and 90 kg DAP/ha, respectively in pot trials at 90 DAS (Table 50) compared to untreated and uninoculated control plants (15.7 cm). Whereas, in field trials, 11 and 9% increase in the length of roots was observed relative to control plants raised in soils treated with 20 kg urea/ha and 90 kg DAP/ha, respectively. Microbial cultures in general, facilitated the root growth more profoundly than the synthetic fertilizers. For instance, the sole application of *R. leguminosarum* among bacterial treatments enhanced the root length considerably by 50% (pot trials) and 16% (field trials) over control plants. Similarly, in the presence of 20 Kg/ha urea was applied to pot trials, the root length was increased maximally by 80, 82 and 73% at 90 DAS in plants bacterized with *P. putida* strain PSE3, *B. pumilus* strain ES3 and *Azotobacter* strain AZ19, respectively, over control. Under field trials receiving 20 kg/ha urea, *P. putida*, *B. pumilus* and *Azotobacter* augmented the root length by 21, 23 and 13%, respectively compare to control at 90 DAS (Table 50, 54, 58). In a similar manner, when *R. leguminosarum* was applied with 90 kg/ha DAP in pot trials, it significantly ($P \leq 0.05$) increased the root length by 80 and 54% after 90 and 120 days of plant growth respectively, compared to uninoculated and untreated control. The coinoculation effects of P-solubilizer, *P. putida*, *B. pumilus* and *Azotobacter* with N-fixer, *R. leguminosarum* on root length of pea plant was also recorded both at 90 and 120 DAS in both pot and field trials and it was found that the root length was significantly ($P \leq 0.05$) increased by 89, 90 and 73% in pot trials after 90 DAS as compare to control. While, 23, 23 and 13% increase in root length was observed when plant was coinoculated with *P. putida, B. pumilus* and *Azotobacter* with *R. leguminosarum,* respectively, after 90 days after sowing in field trials (Table 50, 54 and 58). A similar increase in the length of plant root was observed at 120 DAS when plants were grown in soils treated with or without nitrogenous or phosphatic fertilizers

and bio-primed plants. However, a substantive increase in plant organs was recorded at 120 DAS compared to 90 DAS.

4.9.1.2 Shoot

The shoot length of inoculated/uninoculated plants grown in pot/field soils treated with urea, DAP or mixture of both was variable, when measured at 90 and 120 DAS (Table 48). Among microbial cultures, *Rhizobium* im general showed maximum increase in shoot growth compared to other single application of bacterial cultures and synthetic fertilizers, both in pots and field trials. For example, *R. leguminosarum* when applied alone showed a maximum increase of 31, 18 and 10% over control, 20 kg/ha urea, 90 kg/ha DAP, respectively, in pot experiments. While comparing the effect of fertilizers on shoot growth, it was found that 20 kg/ha urea, 90 kg/ha DAP and mixture of both [urea (20) with DAP (90) kg/ha] enhanced the shoot length by 11, 18 and 47%, respectively, in pot trials. Similarly in field trials, there were 14, 7 and 17% increase in shoot length at 90 DAS following 20 kg/ha urea, 90 kg/ha DAP and mixture of both [urea (20) with DAP (90) kg/ha], application respectively. Generally, when microbial cultures were used with fertilizers, the growth of pea shoots was increased substantially. As an example, in the presence of 20 kg urea/ha urea, *P. putida*, *B. pumilus* and *Azotobacter* considerably increased the shoot length by 13, 18 and 15%, respectively at 90 DAS compared to control. On the contrary, *R. leguminosarum* when used with 90 kg/ha DAP in fields augmented the shoot length by 13% over control at 90 DAS. The shoot length was maximally increased by 89, 91 and 73% respectively as compare to control when plants was coinoculated with P-solubilizers *P. putida* (Table 50), *B. pumilus* (Table 54) and *Azotobacter* (Table 58) with N-fixer *R. leguminosarum* at 90 DAS in pot trials.

4.9.2 Dry matter accumulation

4.9.2.1 Root

Dry matter accumulated within roots of pea growing plants following bio-inoculants and fertilizer application measured at different stages of growth was variable when grown in pot and field conditions (Table 51, 55, 59). The application of N-fertilizer (urea) @20 kg/ha and P-fertilizer (DAP) @ 90 kg/ha and mixture of both fertilizer [urea (20) DAP (90) kg/ha] enhanced the root biomass by 19, 21, and 43% (in pot trials) and 41, 74 and 147%, respectively (in field trials) compared to uninoculated and untreated control at 120 DAS. In comparison, the composite application of fertilizers and microbial inocula, urea with *P. putida*, *B. pumilus* and *Azotobacter*

increased the root dry biomass by 44, 82 and 42%, respectively, in pot trails at 90 DAS compared to un-inoculated and untreated control plants. The sole application of *P. putida B. pumilus*, *Azotobacter* and *R. leguminosarum* enhanced the dry biomass of roots each by 34, 43, 40 and 50%, respectively at 90 DAS compared to control plants. The dry root biomass was increased further by 57, 63, and 54% respectively at 120 DAS (in pot trials) and by 138, 143 and 96%, (in field trials) at 120 DAS when seeds were coinoculated with *P. putida*, *B. pumilus* and *Azotobacter* with N-fixer *R. leguminosarum,* respectively. The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in both pots or in field trials.

4.9.2.2 Shoot

The dry matter accumulation in shoots of inoculated/uninoculated pea plants grown in pot/field soils amended with urea, DAP or mixture of both varied significantly among treatments (Table 51, 55, 59). The single application of P-solubilizers *P. putida*, *B. pumilus*, *Azotobacter* and N-fixer *R. leguminosarum* increased the dry shoot biomass by 54, 29, 54 and 54%, in pot trials and 59, 60, 58 and 13%, respectively in field conditions at 90 DAS which increased even further at 120 DAS compared to un-inoculated and untreated control plants. Among the synthetic fertilizers, the combination of both urea and DAP increased the dry matter accumulation in shoots maximally by 51 and 31% at 90 and 120 DAS, respectively in pot trials. The shoot dry biomass of pea plants raised under field soils was increased considerably by 36, 29 and 138% at harvest (120 DAS) when plants was grown only with 20 kg urea/ha, 90 kg DAP/ha and 20 kg urea/ha with 90 kg DAP/ha, respectively. In comparison, the composite application of fertilizers and microbial inocula (urea with *P. putida* and DAP with *R. leguminosarum*) increased the shoot dry biomass by 54 and 54% in pot trails and 74 and 47% in field conditions respectively, at 90 DAS compared to un-inoculated and untreated control plants. The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials. The dry matter accumulation in whole plants developed either in pots or field soils varied substantially among different treatments.

4.9.2.3 Total dry biomass accumulation

Whole biomass of inoculated pea plants grown in fertilizer amended/untreated pot/field soil invariably differed among treatments. Urea @ 20 kg/ha, DAP @ 90

kg/ha and mixture of both (20 kg/ha urea+90 kg/ha DAP) increased the total dry biomass from 2.35 g/plant (control) to 2.72 g/plant (16%), 2.96 g/plant (26%), and 3.57 g/plant (53%), respectively in pot trials and from 1.49 g/plant (control) to 2.26 g/plant (52%), 2.42 g/plant (62%) and 3.14 g/plant (111%) in field trials at 90 DAS (Fig. 42). Similarly at 120 DAS, the total dry matter accumulation of pea plants grown both in pots and field soils differed among treatments (Fig. 42, 43, 44 and 45). Furthermore, the sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *R. leguminosarum* augmented the dry matter accumulation in plants by 28, 30, 25 and 21%, respectively at 90 DAS compared to control plants. At 120 DAS, the increase in dry matter accumulation in roots was 18 (*P. putida*), 20 (*B. pumilus*), 17 (*Azotobacter*) and 21% (*R. leguminosarum*) in pot experiments (Fig. 42, 44, 46). In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the total dry biomass by 54, 56 and 50%, respectively, in pot trails at 90 DAS while it was 80, 83 and 75% at 120 DAS compared to un-inoculated and untreated control plants. Total dry biomass was increased further by 53, 55, and 50% in pot trials while in field trials this increase was 31, 33 and 32%, at 90 DAS following *R. leguminosarum* inoculation with [*P. putida*], [*B. pumilus*] and [*Azotobacter*], respectively (Fig. 43, 45 and 47). The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials (Fig. 42).

4.9.3 Photosynthetic pigments and symbiotic attributes

The effects of inoculation with [*P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium*] applied alone or in the presence of nitrogenous and phosphatic fertilizers on chlorophyll content in fresh foliage of pea plants differed significantly (Table 52, 56, 60). Among the single-inoculation treatments, *Rhizobium* demonstrated a maximum increase in the chlorophyll content in leaves of pea grown in pots significantly ($P \leq 0.05$) by 35 and 43% above the uninoculated control at 60 DAS, respectively. The dual application of P-solubilizers (*P. putida*, *B. pumilus*, *Azotobacter*) with N_2-fixer (*R. leguminosarum*) in general, further enhanced chlorophyll formation in pea plants developed both in pot and field trials. The co-cultures of *R. leguminosarum* with *P. putida*, *B. pumilus* and *Azotobacter* maximally increased the chlorophyll content in fresh foliage by 51, 55 and 48% (Table 52) respectively as compared to control plants

grown in pot while the chlorophyll content in field crops was increased ($P \leq 0.05$) by 81, 93 and 50% (Table 52), respectively.

4.9.4 Nodulation and leghaemoglobin content

The symbiotic attributes, nodule numbers, nodule dry biomass and leghaemoglobin (Lb) content of pea plants following bio-inoculants [*R. leguminosarum*, *P. putida*, *B. pumilus* and *Azotobacter*] and nitrogenous and phosphatic fertilizer [urea and DAP] application measured after 90 days of growth was variable when grown in pot and field conditions (Table 52, 56, 60). Of the two fertilizers applied both in pots and fields along with/without microbial cultures, DAP (90 kg/ha) had maximum stimulatory effect on chlorophyll formation in pea plants. As an example, in the presence of 90 kg DAP/ha, *R. leguminosarum* maximally enhanced the chlorophyll content from 0.92 mg/g (control) to 1.37 mg/g (49%) in pot grown pea while in field, it increased the chlorophyll content from 0.83 mg/g (control) to 1.7 mg/g (105%), as presented in Table 52. While assessing the impact of fertilizers on chlorophyll synthesis, it was found that there were 21, 26 and 48% increase in chlorophyll contents when plant was grown in soil receiving 20 kg/ha urea, 90 kg/ha DAP and mixture of urea with DAP, respectively. While comparing the impact of fertilizers on measured parameters, the 20 kg urea/ha, 90 kg DAP/ha and mixture of both fertilizers increased the nodule numbers by 34, 28 and 83%, respectively while the Lb content was improved by 20, 34 and 116%, respectively compared to control plants grown in field trials. Among the single-inoculation treatments, *Rhizobium* enhanced the nodule numbers, nodule biomass and leghaemoglobin significantly ($p \leq 0.05$) by 39, 75 and 64% in pot trails while this increase in field trials was 106, 18 and 38%, above the uninoculated control at 90 DAS, respectively. While comparing the impact of all treatments including, sole application of microbial cultures, single dose of each fertilizers and combination of fertilizers and microbes or only the dual inoculation of microbial cultures, it was interesting found that [*R. leguminosarum* with *B. pumilus*] displayed greatest positive impact on the symbiotic attributes of pea plants grown both in pots and field soils. Among the two fertilizers, DAP in general, maximally improved symbiotic characteristics of pea plants when used either alone or in combination with microbial cultures. For example, *Rhizobium* in the presence of 90 kg DAP/ha, enhanced the nodule numbers, nodule dry biomass and Lb content markedly by 73, 85 and 57% in pot trials while under field soils, it increased the measured parameters by 143, 47 and 69%, respectively above the control plants at 90 DAS. Among the combination of microbial cultures, the

composite inoculation of [*Rhizobium* with *Bacillus*] significantly ($p \leq 0.05$) increased the NN, NDB and Lb content in fresh nodules by 86, 91 and 86% (pot experiment) and by 169, 95 and 94% in field grown peas above the control at 90 DAS, respectively.

4.9.5 Concentration and uptake of N and P

Inoculations/fertilizer effects on the uptake and accumulation of N and P by pea plants varied greatly among all treatments (Table 53, 57, 61). *R. leguminosarum*, *P. putida* and *B. pumilus*, and *Azotobacter* used as sole culture, significantly ($p \leq 0.05$) increased the N concentration in roots and shoots by 24 and 9%, 25 and 5%, 29 and 6% and 18 and 4%, at harvest (90 DAS) above the uninoculated control plants grown in pots. Additionally, the *R. leguminosarum*, *P. putida*, *B. pumilus*, and *Azotobacter* applied under field trials significantly ($p \leq 0.05$) enhanced the N contents in roots and shoots of pea plants by 8 and 20%, 7 and 16%, 10 and 18% and, 13 and 8%, respectively above control at harvest. The P concentration in roots and shoots of pea plants grown in pots following sole application of *R. leguminosarum*, *P. putida* and *B. pumilus*, and *Azotobacter* significantly ($p \leq 0.05$) by 42 and 41%, 37 and 34%, 42 and 41% and, 32 and 30%, respectively. Similarly, under field trials, the *R. leguminosarum*, *P. putida*, *B. pumilus*, and *Azotobacter* significantly enhanced the P contents in roots and shoots of pea plants by 14 and 48%, 10 and 26%, 24 and 35% and, 5 and 17%, respectively, relative to control at harvest. Among fertilizers, DAP had maximum positive effect and increased the root N (35 mg/g), root P (0.25mg/g) and shoot P (0.3 mg/g) by 26, 24 and 32%, respectively whereas the shoot N (34.7 mg/g) was maximally increased by 26% when pea plants was grown in soil treated with urea as compared to control plants grown in pot trials. *R. leguminosarum* inoculated plants grown in soil treated with DAP increased the N contents in roots and shoots and P contents in roots and shoots by 24, 11, 89 and 33% in pot trials and 20, 28, 38 and 47% in field conditions, respectively over non inoculated and non-treated control plants (Table 53). Similarly, the mixture of both urea (20 kg/ha) and DAP (90 kg/ha) showed maximum increase in the measured parameters compared to other single treatment of urea or DAP or control plants in both pot and field trials. Of the two fertilizers, DAP in general, had maximum positive effect on the measured parameters of either inoculated or un-inoculated pea plants. While comparing the impact of single microbial cultures on N and P contents in roots and shoots of pea

plants grown both in pots and field soils, it was observed that *Rhizobium* had the largest stimulatory effect on N and P concentration (Table 53). In a similar way, when effect of single application of microbial cultures was compared with those of urea and DAP used alone, it was found that microbial cultures in general had better effect on N and P concentration in both roots and shoots of pot and field grown greengram plants. The co-cultures of *R. leguminosarum* and *B. pumilus* showed the largest increment of 36 and 43 (N contents), 89 and 88% (P contents) in pot trials and 37 and 50 (N contents), 71 and 70% (P contents) in field conditions respectively in roots and shoots compared to control.

4.9.6 Seed yield and seed protein

The single or composite inoculation effects of [*P. putida*, *B. pumilus*, *Azotobacter* and *R. leguminosarum*], applied alone or in the presence of nitrogenous and phosphatic fertilizers on seed yield and seed protein of pea plants varied among treatments (Table 53, 57 and 61). Of the sole-inoculations, *R. leguminosarum* demonstrated a maximum increase in the seed yield of pea grown in pots significantly ($P \leq 0.05$) by 55 (6.5 g/plant) and 52% (8.2 g/plant) above the uninoculated control (pot: field = 4.2:5.4 g/plant) at harvest. Microbial cultures in general, substantially augmented the seed yield when applied with any of the test fertilizers in both pot and field soil. For instance, in the presence of 20 kg urea/ha, *B. pumilus* among microbial cultures was found to exhibit maximum increase in the seed yield, both in pot trials (95%) and field environment (76%) while *R. leguminosarum* in the presence of 90 kg DAP/ha augmented the seed yield by 79 (pot trials) and 66% (field) compared to untreated and uninoculated plants. Moreover, the combination of *R. leguminosarum* strain RP2 with [(i) *P. putida* strain PSE3 (ii) *B. pumilus* strain ES3 and (iii) *Azotobacter* strain AZ19] increased the seed yield by 123, 126 and 116% (in pot trials) and 89, 95 and 82% (field trials), respectively. The mixed application of recommended dose of urea (20 kg/ha) and DAP (90 kg/ha) enhanced the seed production from 4.2 g/plant (control) to 8.4 g/plant in pots while it changed from 5.4 g/plant (control) to 9.2 g/plant in field conditions. In addition, microbial cultures applied with fertilizers augmented the seed yield even further both in pots and field soils. The protein content in grains even-though did not differ significantly among treatments yet it was greater in inoculated plants than the control ones (Table 51, 55, 59). Among all treatments, the co-culture of [*Bacillus* and *Rhizobium*] enhanced the grain protein from 275 mg/g (control) to 279 in pots and 272 mg/g (control) to 294 mg/g of seed in field grown peas. The two

way ANOVA in general revealed that the individual effect of inoculants (df=3), further application of fertilizer (df=1) and interaction between inoculation and fertilizer (df=3) was significant (P≤0.05) for all measured parameters.

Greengram

4.10.1 Length of plant organs

4.10.1.1 Root

The inoculated and uninoculated greengram plants grown in soils supplemented with/without recommended rates of fertilizers had variable plant growth. The length of roots was increased by 36 (25.2 cm) and 40% (25.9 cm) when plants were grown in soil treated with 25 kg urea/ha and 85 kg DAP/ha, respectively in pot trials after 50 DAS (Table 62) compared to uninoculated and untreated control plants (18.5 cm). The length of roots were increased even further by 75 (pot trials) and 95% (field trials) when plants were grown in soil treated with [25 kg urea/ha with 85 kg DAP/ha] at 50 DAS relative to control. Likewise, the root length increased substantially when greengram was grown under field trials. Among all single microbial inoculations, [*Bradyrhizobium* (sp.) vigna] in particular showed a maximum increase of 60 and 76% in root length of pot and field grown greengram uprooted at 50 DAS relative to control plants. The root length was however, substantially increased by 68, 74 and 70% after 50 days of growth when P. *putida* strain PSE3, *B. pumilus* strain ES3 and *Azotobacter* strain AZ19 bacterized seeds, respectively, were grown in pot soils treated with 25 kg urea/ha. In field trials, the *P. putida*, *B. pumilus* and *Azotobacter* inoculated greengram plants grown in soils treated with 25 kg/ha urea had 84, 93 and 76% more root growth, respectively, compared to control at 50 DAS. In a similar experiment, when 85 kg/ha DAP was applied together with *Bradyrhizobium* in field trials the root length was increased by 82 (33.2 cm) and 64% (34.5 cm) after 50 and 80 days of growth, respectively over uninoculated and untreated control plants. The coinoculation effect of P-solubilizer, *P. putida*, *B. pumilus* and *Azotobacter* with symbiotic N-fixer, *Bradyrhizobium* on root length of greengram plant observed at seed formation (80 50 DAS) and at harvest (80 DAS) in both pot and field trials was variable and it was significantly (P≤0.05) increased by 81 [*P. putida* with *Bradyrhizobium*], 90 [*B. pumilus* with *Bradyrhizobium*] and 77% [*Azotobacter* with *Bradyrhizobium*] in pot trials after 50 DAS as compare to control. While, there were 100, 110 and 97% increase in root length when plants were coinoculated with [*P. putida* with *Bradyrhizobium*] (Table 62) [*B. pumilus* with *Bradyrhizobium*] (Table 66)

and [*Azotobacter* with *Bradyrhizobium*] (Table 70) respectively, 50 days after sowing greengram in field trials. A similar increase in the length of roots detached from bio-primed plants was observed at 80 DAS when such plants were grown in soils treated with or without nitrogenous or phosphatic fertilizers. However, a substantive increase in plant organs in general, was recorded at 80 DAS compared to 50 DAS.

4.10.1.2 Shoot

The shoot length of inoculated or uninoculated plants grown in pot/field soils amended with/without urea and DAP applied either alone or as mixture, measured at 50 and 80 DAS was variable. Among single inoculation treatments, *B. pumilus* showed maximum increase (46%) in shoot length in pot experiments while [*Bradyrhizobium* (sp.) vigna] substantially enhanced the shoot length by 56% in field trials (Table 66) at 50 DAS compared to control. Furthermore, the shoot length was increased by 19, 26 and 56% at 50 DAS, when plants were grown in pot soils supplemented with 25 kg/ha urea, 85 kg/ha DAP and mixture of [25 kg/ha urea with 85 kg/ha DAP], respectively, compared to control. At 25 kg/ha urea, 85 kg/ha DAP and mixture of 25 kg/ha urea with 85 kg/ha DAP, applied in field trials, this increase was found as 29, 39 and 70%, respectively, at 50 DAS in comparison to control. When *P. putida*, *B. pumilus* and *Azotobacter* seeds were sown and grown in pot soils amended with 25 kg urea/ha urea, the shoot length was found to increase significantly ($P \leq 0.05$) by 46 (33.4 cm), 54 (35.3 cm) and 48% (33.8 cm) respectively, at 50 DAS compared to control plants. In contrast, at 85 kg/ha DAP, applied to field soils, the shoot length of the *Bradyrhizobium* inoculated greengram increased by 53% as compared to control plants 50 days after sowing. The shoot length was maximally and significantly ($P \leq 0.05$) increased by 81, 90 and 77% when plants was coinoculated with P-solubilizers [*P. putida* with *Bradyrhizobium*], [*B. pumilus* with *Bradyrhizobium*] and [*Azotobacter* with N-fixer *Bradyrhizobium*] at 50 DAS in pot trials as compare to control (Table 62, 66, 70). In general, the application of phosphatic fertilizer (DAP) used either alone or as mixture with *Bradyrhizobium*, had more pronounced impact on root and shoot growth of greengram plants grown either in pot or field environment. While comparing the effect of fertilizers (nitrogenous and phosphatic), inocula or culture with fertilizers, the combined application of urea and DAP was found superior over all treatments except the dual inoculation of *Bradyrhizobium* with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter*.

4.10.2Dry matter accumulation

4.10.2.1 Root

Recommended rates of urea (25 kg/ha) and DAP (85 kg/ha) and mixture of both significantly ($P \leq 0.05$) increased the dry root biomass by 17 (0.95 g/plant), 26 (1.02 g/plant), and 53% (1.24 g/plant) in pot trails and 28 (0.96 g/plant), 37 (1.03 g/plant) and 80% (1.24 g/plant) (field trials) at 50 DAS compared to control plants (pots: field= 0.81:0.75 g/plant). The sole application of P-solubilizers *P. putida*, *B. pumilus*, *Azotobacter* and N-fixer *Bradyrhizobium* sp. (vigna) increased the dry root biomass from 0.81 g/plant to 1.12 g/plant (38%), 1.16 g/plant (43%), 1.08 g/plant (33%) and 1.15 g/plant (42%), in pot trials and from 0.75 g/plant to 1.14 g/plant (52%), 1.18 g/plant (57%), 1.11 g/plant (43%) and 1.15 g/plant (53%), respectively in field conditions at 50 DAS. The root biomass of both inoculated and fertilizer applied plants increased even further at 80 DAS compared to control plants (Table 61, 65, 69). In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the root dry biomass by 46, 49 and 42%, respectively, in pot trails at 50 DAS compared to un-inoculated and untreated control plants. While comparing the impact of mixed inocula on the development of root system, it was observed that the dry root biomass was increased significantly ($P \leq 0.05$) by 63, 69, and 58% in pot trials while in field trials this increase was 80, 85 and 73%, at 50 DAS following [*P. putida* with *Bradyrhizobium*] (Table 63), [*B. pumilus* with *Bradyrhizobium*] (Table 67) and [*Azotobacter* with *Bradyrhizobium*] (Table 71) respectively.

4.10.2.2 Shoot

The dry matter accumulation in shoots of greengram plants growing in soils (pot and field) treated with urea, DAP or both and inoculated or coinoculated with *P. putida*, *B. pumilus* and *Azotobacter* with *Bradyrhizobium* differed profoundly both at 50 and 80 DAS (Table 63, 67 and 71). The single application of *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* increased the dry shoot biomass in greengram plants by 36 (1.31 g/plant), 39 (1.33 g/plant), 34 (1.29 g/plant) and 33% (1.28 g/plant) in pot experiments and by 47 (1.34 g/plant), 49 (1.36 g/plant), 43 (1.3 g/plant) and 41% (1.28 g/plant), respectively in field conditions at 50 DAS which was increased even further at 80 DAS compared to un-inoculated and untreated control plants. The shoot dry biomass of greengram plants raised under field soils was increased considerably by 28, 37 and 65% at 50 DAS when plants was grown only with 25 kg urea/ha, 85 kg

DAP/ha and 25 kg urea/ha with 85 kg DAP/ha, respectively. While, the combination of both urea and DAP increased the dry matter accumulation in shoots maximally by 39 and 36% compared to control at 50 and 80 DAS, respectively in field trials. In comparison, the composite application of fertilizers and microbial inocula increased the shoot dry biomass by 52 (urea with *P. putida*), 55 (urea with *B. pumilus*), 47 (urea with *Azotobacter*), 55% (DAP with *Bradyrhizobium*) in pot trails and 59 (urea with *P. putida*), 65 (urea with *B. pumilus*), 53 (urea with *Azotobacter*) and 64% (DAP with *Bradyrhizobium*) in field conditions, respectively, at 50 DAS compared to un-inoculated and untreated control plants. The dry shoot biomass was increased further by 63, 69, and 58% in pot trials while in field trials this increase was 65, 68 and 60%, at 50 DAS following [*P. putida* with *Bradyrhizobium*], [*B. pumilus* with *Bradyrhizobium*], and [*Azotobacter* with *Bradyrhizobium*].

4.10.2.3 Total dry biomass accumulation

In this experiment, the recommended rates of urea and DAP and mixture of both fertilizers enhanced the whole dry biomass from 1.77 g/plant (control) to 2.16 g/plant (22%), 2.27 g/plant (28%), and 2.78 g/plant (57%), respectively in pot trials and from 1.77 g/plant (control) to 2.16 g/plant (31%), 2.27 g/plant (38%) and 2.78 g/plant (67%) in field trials at 50 DAS. Similarly at 80 DAS, the total dry matter accumulation of greengram plants grown both in pots and field soils differed among treatments (Fig. 48). Furthermore, the sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* augmented the dry matter accumulation in plants by 37, 41, 32 and 37%, respectively at 50 DAS compared to control plants. At 80 DAS, the increase in dry matter accumulation in roots was 33 (*P. putida*), 36 (*B. pumilus*), 31 (*Azotobacter*) and 33% (*Bradyrhizobium*) in pot experiments (Fig. 48, 50, 52). In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the total dry biomass by 49, 52 and 45%, respectively, in pot trails at 50 DAS while it was 46, 49 and 42% at 80 DAS compared to un-inoculated and untreated control plants. Total dry biomass was increased further by 64, 68, and 59% in pot trials while in field trials this increase was 77, 82 and 72%, at 50 DAS following *Bradyrhizobium* inoculation with [*P. putida*], [*B. pumilus*] and [*Azotobacter*], respectively (Fig. 49, 51, 53). The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials (Fig. 49).

4.10.3 Photosynthetic pigments and symbiotic attributes

The effects of inoculation with [*Bradyrhizobium*, *P. putida*, *B. pumilus* and *Azotobacter*], applied alone or in the presence of nitrogenous and phosphatic fertilizers on chlorophyll content in fresh foliage of greengram plants differed significantly (Table 64, 68, 72). While assessing the impact of fertilizers on chlorophyll synthesis, it was found that there were 11, 7 and 34% increase in chlorophyll contents when plant was grown in pot soil receiving 25 kg/ha urea, 85 kg/ha DAP and mixture of urea with DAP, respectively. Among the single-inoculation treatments, *Bradyrhizobium* had maximum stimulatory effect on chlorophyll formation in foliage of greengram plants and increased it significantly ($P \leq 0.05$) by 17 and 20% when greengram plants were maintained in pots and fields, above the uninoculated control at 50 DAS, respectively. The impact of *Bacillus* on chlorophyll synthesis in greengram plants grown both in pots and fields was statistically at par with *Bradyrhizobium* effect. Of the two fertilizers applied both in pots and fields along with/without microbial cultures, DAP (85 kg/ha) demonstrated the largest positive effect on chlorophyll formation in greengram plants. As an example, in the presence of 85 kg DAP/ha, *Bradyrhizobium* maximally enhanced the chlorophyll content from 0.83 mg/g (control) to 1.09 mg/g (31% increase) in pot grown greengram while in field, the chlorophyll content changed from 0.89 mg/g (control) to 1.13 mg/g (34% increase), as presented in Table. The dual application of P-solubilizers (*P. putida*, *B. pumilus*, *Azotobacter*) with N-fixer [*Bradyrhizobium* sp. (vigna)] in general, further enhanced chlorophyll formation in greengram plants developed both in pot and field trials. The co-cultures of *Bradyrhizobium* with [*P. putida*], [*B. pumilus*] and [*Azotobacter*] maximally increased the chlorophyll content in fresh foliage by 35, 37 and 33% (Table 64, 68 and 72) respectively as compared to control plants grown in pot while the chlorophyll content in field crops was increased ($P \leq 0.05$) by 37, 39 and 34% respectively.

4.10.4 Symbiotic characteristics: Nodulation and leghaemoglobin content

The symbiotic attributes including nodule formation and leghaemoglobin accumulation with nodules of greengram plants due to bacterial inoculation [*Bradyrhizobium*, *P. putida*, *B. pumilus* and *Azotobacter*] and fertilizer [urea and DAP] application recorded 50 days after growth differed invariably among treatments both in pots and field conditions (Table 64, 68, 72). Among the tested fertilizers, 25 kg urea/ha, 85 kg DAP/ha and mixture of both fertilizers enhanced the nodule numbers by 42 (34 nodules/plant), 46 (35 nodules/plant) and 113% (51 nodules/plant), respectively

compared to control (24 nodules/plant) while the Lb content in fresh nodules removed at 50 DAS, was improved by 50 [0.12mM $(gfm)^{-1}$], 50 [0.12mM $(gfm)^{-1}$], and 100% [0.16mM $(gfm)^{-1}$], respectively compared to control plants [0.08 mM $(gfm)^{-1}$] grown in pot trials. Similarly, the single or mixed application of fertilizers profoundly increased the measured parameters under field soils (Table 64, 68, 72). Among the single-inoculation treatments, *Bradyrhizobium* had the largest positive effect and enhanced the nodule numbers, nodule dry biomass and Lb content significantly ($p \leq 0.05$) by 117 (52 nodules/plant), 122 (191 mg/plant) and 113% [0.17mM $(gfm)^{-1}$] in pot trails while this increase in field trials was 162 (55 nodules/plant), 155 (191 mg/plant) and 143%, [0.17mM $(gfm)^{-1}$] above the uninoculated control at 50 DAS, respectively. Similarly, microbial cultures in general when used with fertilizers facilitated the formation of nodules on the root systems of greengram plants compared to the sole application of urea or DAP. As an example, the nodule numbers, nodule dry biomass, and Lb content of *Bradyrhizobium* inoculated greengram plants grown with 85 kg DAP/ha in pots were found to be enhanced by 133 (56 nodules/plant), 134 (201 mg/plant), and 125% [0.18mM $(gfm)^{-1}$] over control which was statistically at par with the combined application of *Bacillus* and urea Table). The formation and distribution of nodules and lb content in fresh nodules detached from the inoculated or uninoculated greengram plants measured at podfill stage of growth was also variable in field conditions. Generally, the combined application of N-fixer and P-solubilizer showed a prolific impact on symbiotic attributes of greengram plants compared to other single or simultaneous application of bacterial cultures. For example, the co-culture of *Bradyrhizobium* with *B. pumilus* significantly ($P \leq 0.05$) increased the nodule numbers, nodule dry biomass and Lb contents in fresh nodules by 179 (67 nodules/plant), 190 (249 mg/plant) and 200% [0.18mM $(gfm)^{-1}$] (pot trials) and by 229 (69 nodules/plant), 248 (261 mg/plant) and 243% [0.24mM $(gfm)^{-1}$] (field trials) compared to control plants.

4.10.5 Concentration and uptake of N and P

The N and P uptake by greengram plants following microbial/fertilizer application varied considerably among treatments (Table 65, 69, 73). Among fertilizers, DAP had maximum positive effect and increased the root N (41 mg/g), root P (0.32mg/g) and shoot P (0.39 mg/g) by 28, 45 and 41%, respectively whereas the shoot N (59 mg/g) was maximally increased by 23% when greengram plants was grown in pot soils.

Single inoculation with *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* significantly increased the N contents by 38 and 29% (root:shoot), 44 and 33% (root:shoot), 28 and 23% (root:shoot) and 44 and 35% (root:shoot) at 80 DAS compared to the control plants grown in pots. Similarly, under field trials, the *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* significantly enhanced the N contents in roots and shoots of greengram plants by 61 and 41%, 64 and 45%, 54 and 36% and, 68 and 52%, respectively. The P concentration in roots and shoots of greengram plants grown in pots following sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* was significantly ($P \leq 0.05$) by 64 and 44%, 73 and 53%, 55 and 31% and, 55 and 41%, respectively. Similarly, under field trials, the *P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium* significantly ($P \leq 0.05$) enhanced the P contents in roots and shoots of greengram plants by 85 (0.37mg/g) and 66% (0.48 mg/g), 95 (039 mg/g) and 76% (0.51 mg/g), 75 (0.35 mg/g) and 52% (0.44 mg/g) and 70 (0.34 mg/g) and 55% (0.45 mg/g), respectively. While comparing the impact of single microbial cultures on N and P contents in roots and shoots of greengram plants grown both in pots and field soils, it was observed that *B. pumilus* in general, had the largest stimulatory effect on N and P concentration (Table 69). *Bradyrhizobium* inoculated plants grown in soil treated with DAP increased the N contents in roots and shoots and P contents in roots and shoots by 56, 39, 91 and 53% in pot trials and 89, 54, 110 and 79% in field conditions, respectively over non inoculated and non- treated control plants (Table 73). Similarly, the mixture of both urea (25 kg/ha) and DAP (85 kg/ha) showed maximum increase in the measured parameters compared to other single treatment of urea or DAP or control plants in both pot and field trials (Table 69). In a similar way, when effect of single application of microbial cultures was compared with those of urea and DAP used alone, it was found that microbial cultures in general had better effect on N and P concentration in both roots and shoots of pot and field grown greengram plants. The co-inoculation of [*Bradyrhizobium* and *B. pumilus*] displayed even though greatest positive effect on N and P accumulation within roots and shoots but the impact on both pot and field grown greengram was marginally different.

4.10.6 Seed yield and seed protein

Urea and DAP applied independently both in pots and field had a variable effect on seed production in greengram plants (Table 65, 69 and 73). However, there were no significant differences in seed yield of greengram when grown either in the presence

of urea or DAP applied to both pot and field soil. Among microbial treatments, the single inoculation with [*P. putida*, *B. pumilus*, *Azotobacter* and *Bradyrhizobium*] significantly ($P \leq 0.05$) increased the seed yield in pot experiments by 69 (5.4g/plant), 75 (5.6 g/plant), 56 (5 g/plant) and 59% (5.1 g/plant) relative to the control (3.2 g/plant), as presented in Table 65, 69 and 73. While comparing the effect of fertilizers and sole application of bacterial cultures, *Bacillus* among microbial treatments showed maximum increase in seed yield over 25 kg/ha (25% increase) and DAP (22% increase). Moreover, in the presence of 25 kg urea/ha, *B. pumilus* had the maximum positive effect on seed formation in greengram and increased it significantly ($P \leq 0.05$) by 94% while *Bradyrhizobium* in the presence of 85 kg DAP/ha augmented the seed yield by 90% compared to untreated and uninoculated plants grown in pot experiments. Under field trials, *Bradyrhizobium* in the presence of 85 kg DAP/ha increased the seed yield significantly ($P \leq 0.05$) by 103% compared to control. The dual inoculation of [*B. pumilus* and *Bradyrhizobium*] increased the grain yield maximally by 128% (7.3 g/plant) and 121% (7.5 g/plant) respectively in pot and field trials. Mixed application of recommended dose of urea (25 kg/ha) and DAP (85 kg/ha) increased the seed yield by 103% and 109% in pot and field conditions, respectively over control. While comparing the effect of best performing combination [*B. pumilus* and *Bradyrhizobium*] and mixture of fertilizers [25 kg urea/ha+85 kg DAP/ha], it was found that the combination of cultures gave a significant increase of 11% over mixed application of fertilizers. The protein content in greengram seeds even-though did not differ significantly among treatments yet it was greater in inoculated plants compared to control ones (Table 69) grown both in pot and field experiments. The increase in grain protein however ranged between 3 (fertilizer) to 8% (*Bacillus* with urea/*Bradyrhizobium*) in pots while in field the increase in grain protein varied between 4 to 9%. The two way ANOVA in general revealed that the individual effect of inoculants (df=3), further application of fertilizer (df=1) and interaction between inoculation and fertilizer (df=3) was significant ($P \leq 0.05$) for all measured parameters.

Lentil

4.11.1 Length of plant organs

4.11.1.1 Root

In this experiment, the inoculated and uninoculated lentil plants grown in soils treated with or without basal dose of synthetic chemical fertilizers showed variable biological

properties. The length of roots of uninoculated plants was increased by 20 (18.1 cm) and 22% (17.8 cm) when plants were grown in soil treated with 30 kg urea/ha and 90 kg DAP/ha, respectively, in pot trials at 90 DAS (Table 74) compared to control plants (14.8 cm) grown in the absence of urea and DAP. At 120 DAS, the length of roots further enhanced by 31 and 36% when plants were grown in soil treated with 30 kg urea/ha and 90 kg DAP/ha, respectively, relative to control. There was considerable increase in the root length of lentil plants both at 90 and 120 DAS when grown under field trials. Moreover, the root length was substantially increased by 47, 54 and 47% after 90 days of growth when P. *putida* strain PSE3, *B. pumilus* strain ES3 and *Azotobacter* strain AZ19 bacterized seeds, respectively, were grown in pot soils treated with 30 kg urea/ha. In field trials, *B. pumilus* inoculated lentil plants grown in soils treated with 30 kg/ha urea had the greatest root growth both at 90 (76% more than control) and at 120 DAS compared to control (Table 74, 78 and 82). In a similar experiment, when 90 kg/ha DAP was applied together with *Rhizobium* in field trials the root length was increased by 67 (28.7 cm) and 56% (30.2 cm) after 90 and 120 days of growth, respectively over uninoculated and untreated control plants. The coinoculation effect of P-solubilizer, *P. putida*, *B. pumilus* and *Azotobacter* with symbiotic N-fixer, *Rhizobium* on root length of lentil plant observed at 90 and 120 DAS in both pot and field trials was variable and it was significantly ($P \leq 0.05$) increased by 70, 76 and 61% in pot trials after 90 DAS as compare to control. While, a maximum of 83, 90 and 79% increase in root length was recorded when plant was coinoculated with *P. putida*, *B. pumilus* and *Azotobacter* with *Rhizobium,* respectively, 90 days after sowing in field trials. A similar increase in the length of plant root was observed at 120 DAS when plants were grown in soils treated with or without nitrogenous or phosphatic fertilizers and bio-primed plants. However, a substantive increase in plant organs was recorded at 120 DAS compared to 90 DAS.

4.11.1.2 Shoot

The shoot length of inoculated or uninoculated plants grown in pot/field soils receiving urea and DAP applied either alone or as mixture was recorded at 90 and 120 DAS. The shoot length was increased by 23, 35 and 65% when plants were grown in pot soils supplemented with 30 kg/ha urea, 90 kg/ha DAP and mixture of 30 kg/ha urea with 90 kg/ha DAP, respectively, compared to control. At 30 kg/ha urea, 90 kg/ha DAP and mixture of 30 kg/ha urea with 90 kg/ha DAP, applied in field trials, this increase was found as 25, 31 and 47%, respectively, at 90 DAS in comparison to

control. When *P. putida*, *B. pumilus* and *Azotobacter* seeds were sown and maintained in pot soils amended with 30 kg urea/ha, the shoot length was found to increase by 58 (29.3 cm) (Table 74), 65 (30.5 cm) (Table 78) and 55% (28.7 cm) (Table 82) respectively, at 90 DAS compared to control plants. In contrast, at 90 kg/ha DAP, applied to field soils, the shoot length of the *Rhizobium* inoculated lentil increased by 48% as compared to control plants 90 days after sowing. The shoot length was maximally increased by 69, 75 and 64% respectively as compare to control when plants was co-inoculated with *P. putida*, *B. pumilus* and *Azotobacter* with *Rhizobium* at 90 DAS in pot trials. Among the co-inoculation treatments, the dual cultures of [*Pseudomonas* with *Rhizobium*] used independently enhanced the shoot length by 68% in pots while this increase was 42% in field soils over control plants analyzed at 120 DAS. In general, the application of phosphatic fertilizer (DAP) used either alone or as mixture with *Rhizobium*, had more pronounced impact on root and shoot growth of lentil plants grown either in pot or field environment. While comparing the effect of fertilizers (nitrogenous and phosphatic), inocula or culture with fertilizers, the combined application of urea and DAP was found superior over all treatments except the dual inoculation of *Rhizobium* with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter*.

4.11.3 Dry matter accumulation

4.11.3.1 Root

The application of 30 kg urea/ha and 90 kgDAP/ha and mixture of both fertilizers (30 kg/ha urea+90 kg/ha DAP) increased the dry root biomass from 0.52 g/plant (control) to 0.61 g/plant (17%), 0.63 g/plant (21%), and 0.71 g/plant (37%), respectively in pot trials and from 0.62 g/plant (control) to 0.78 g/plant (26%), 0.81 g/plant (31%) and 1.03 g/plant (66%) in field trials at 90 DAS. Similarly at 120 DAS, the dry matter accumulation within roots of lentil plants grown both in pots and field soils differed among treatments (Table 75). Furthermore, the sole application of *P. putida, B. pumilus*, *Azotobacter* and *Rhizobium* augmented the dry matter accumulation in roots by 29, 33, 23 and 31%, respectively at 90 DAS compared to control plants. At 120 DAS, the increase in dry matter accumulation in roots was 26 (*P. putida*), 26 (*B. pumilus*), 22 (*Azotobacter*) and 28% (*Rhizobium*) in pot experiments. In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the root dry biomass by 50, 49 and 46%, respectively, in pot trails at 90 DAS while it was 32, 33 and 25% at 120

DAS compared to un-inoculated and untreated control plants. The dry root biomass was increased further by 42, 46, and 37% in pot trials while in field trials this increase was 81, 85 and 77%, at 90 DAS following *Rhizobium* inoculation with [*P. putrida*], [*B. pumilus*] and [*Azotobacter*], respectively (Table 75, 79 and 83). The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials.

4.11.3.2 Shoot

The dry matter accumulation in shoots of lentil plants following inoculation with/without *P. putida*, *B. pumilus* and *Azotobacter,* and *Rhizobium* differed profoundly both at 90 and 120 DAS (Table 75, 79, 83). Generally, the mixture of fertilizers had maximum positive effect on shoot biomass of lentil plants grown both in pots and field at 90 and 120 DAS, over control or single application of each fertilizer. As an example, the combination of both urea (30 kg/ha) and DAP (90 kg/ha) increased the dry matter accumulation in shoots significantly ($P \leq 0.05$) by 66 (90 DAS) and 71% (120 DAS) in pots and 62 (90 DAS) and 79% (120 DAS) in field compare to control. Similarly, the combined effect of the two fertilizers was 24 (90 DAS) and 25 (120 DAS) greater than 30 kg urea/ha applied in pot experiment while the increment in field grown plants was 17 (90 DAS) and 23% (120 DAS). The single application of P-solubilizers *P. putida*, *B. pumilus*, *Azotobacter* and symbiotic N-fixer *Rhizobium* increased the dry shoot biomass in lentil plants by 38, 41, 35 and 41% (in pot trials) and by 50, 52, 49 and 52%, respectively (in field conditions) at 90 DAS which was increased even further at 120 DAS compared to un-inoculated and untreated control plants. In comparison, the composite application of fertilizers and microbial inocula increased the shoot dry biomass by 56 (urea with *P. putida)* and 61% (DAP with *Rhizobium*) in pot trails and 59 (urea with *P. putida*) and 61% (DAP with *Rhizobium*) in field conditions, respectively, at 90 DAS compared to un-inoculated and untreated control plants. The dual cultures of [*Bacillus* with *Rhizobium*] enhanced the shoot dry biomass by 79 (90 DAS) and 83 (120 DAS) in pot trials where as in field experiments the shoot biomass was increased by 71 (90 DAS) and 86% (120 DAS) relative to control. Additionally, the application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field soils.

The dry matter accumulation in whole plants detached from both pot and field experiments varied substantially among different treatments.

4.11.2.3 Total dry biomass accumulation

Total dry matter accumulated in lentil plants following bio-inoculants and fertilizer application measured at different stages of growth was variable when grown in pot and field conditions. The application of N-fertilizer (urea) @ 30 kg/ha and P-fertilizer (DAP) @ 90 kg/ha and mixture of both fertilizers (30 kg/ha urea+90 kg/ha DAP) increased the total dry biomass from 1.73 g/plant (control) to 2.23 g/plant (29%), 2.28 g/plant (32%), and 2.72 g/plant (57%), respectively in pot trials and from 1.96 g/plant (control) to 2.63 g/plant (34%), 2.77 g/plant (41%) and 3.32 g/plant (63%) in field trials at 90 DAS. Similarly at 120 DAS, the total dry matter accumulation of lentil plants grown both in pots and field soils differed among treatments (Fig. 55, 56). Furthermore, the sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* augmented the dry matter accumulation in plants by 35, 39, 32 and 40%, respectively at 90 DAS compared to control plants. At 120 DAS, the increase in dry matter accumulation in roots was 37 (*P. putida*), 35 (*B. pumilus*), 40 (*Azotobacter*) and 54% (*Rhizobium*) in pot experiments. In comparison, the composite application of fertilizers and microbial inocula for example, urea with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* enhanced the total dry biomass by 49, 51 and 45%, respectively, in pot trails at 90 DAS while it was 54, 56 and 49% at 120 DAS compared to un-inoculated and untreated control plants (Fig. 54, 56 and 58). Total dry biomass was increased further by 65, 69, and 59% in pot trials while in field trials this increase was 72, 76 and 70%, at 90 DAS following *Rhizobium* inoculation with [*P. putida*], [*B. pumilus*] and [*Azotobacter*], respectively (Fig. 55, 57 and 59). The application of fertilizers used either alone or in combination with bacterial inoculants consistently increased the measured parameters with increase in plant age when grown in pot or field trials (Fig. 54 and 55).

4.11.4 Photosynthetic pigments and symbiotic attributes

The chlorophyll content in fresh foliage of lentil plants was increased by 21, 29 and 50% (pot trials) and 23, 35 and 86% (field trials) when plant was gown in soils receiving 30 kg/ha urea, 90 kg/ha DAP and mixture of urea with DAP respectively. In the presence of 90 kg DAP/ha, *Rhizobium* enhanced the chlorophyll content from 0.78 mg/g (control) to 1.14 mg/g in pot while in field, it increased the chlorophyll content from 0.75 mg/g (control) to 1.24 mg/g (Table 76, 80, 84). The chlorophyll content

was increased by 32, 35, 28 and 35% respectively in fresh foliage of lentil plants inoculated with sole application of *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* in pot trials. The dual application of P-solubilizers (*P. putida*, *B. pumilus*, *Azotobacter*) with N-fixer (*Rhizobium*) in general, had maximum positive impact on chlorophyll formation in pot and field trials. The co-cultures of *Rhizobium* with *P. putida* (Table 76) *B. pumilus* (Table 80) and *Azotobacter* (Table 84) maximally increased the chlorophyll content in fresh foliage significantly ($P \leq 0.05$) by 55, 58 and 53%, respectively as compared to control plants grown in pots while in field, it was 71, 75 and 67%, respectively.

4.11.5 Nodulation and leghaemoglobin content

The formation of nodules onto the root systems of inoculated or uninoculated pot and field grown lentil plants measured at podfill stage of growth was variable. Urea at 30 kg /ha, DAP at 90 kg/ha and mixture of both fertilizers increased the nodule numbers by 17 (21 nodules/plant), 22 (22 nodules/plant) and 61% (29 nodules/plant), respectively, while the Lb content was improved by 13 [0.17 mM $(gfm)^{-1}$], 27 [0.19 mM $(gfm)^{-1}$] and 67% [0.25 mM $(gfm)^{-1}$], respectively compared to control [0.15 mMgfm^{-1}] plants grown in pot trials (Table 76). Generally, the single or mixed inoculation of *Rhizobium* had a remarkable impact on nodulation compared to other single or simultaneous application of bacterial cultures. For example, the nodule numbers, nodule dry biomass and Lb content in fresh nodules were increased significantly ($P \leq 0.05$) by 56, 50 and 67% in pot trails while this increase in field trials was 64, 75 and 107%, respectively following single inoculation of *Rhizobium* only over uninoculated but untreated control plants. Also, bacterial cultures in general resulted in greater nodulation onto the root systems of lentil plants compared to urea or DAP application. For example, *B. pumilus* among inoculants markedly enhanced the nodule numbers, nodule dry biomass and Lb content by 50, 46 and 60% under pot environment and by 59, 70 and 100%, in field soils, respectively, over control (Table 80). Among the two fertilizers, DAP in general, resulted in more nodule formation when used either alone or in combination with microbial cultures. As an example, the nodule numbers, nodule dry biomass and Lb content was increased in field grown lentil plants by 77, 87, and 138% when *Rhizobium* was used with 90 kg DAP/ha over control. The coinoculation of N-fixer *Rhizobium* with- (i) *P. putida* (ii) *B. pumilus* and (iii) *Azotobacter* increased the nodule numbers, nodule dry biomass and Lb content by- (i) 72 (31 nodules/plant), 64 (312 mg/plant) and 64% [0.29 mM $(gfm)^{-1}$] (ii) 58 (33 nodules/plant) 83 (327 mg/plant) and 72% (0.31 mMgfm^{-1}} and (iii) 61 (29 nodules/plant), 58 (300 mg/plant) and 67%

(0.25 mM $(gfm)^{-1}$) compared to plants raised in pot soil. The nodule biomass of other inoculated and uninoculated plants followed a trend similar to those observed for nodule formations.

4.11.5 Concentration and uptake of N and P

The influence of bacterial inoculation or fertilizer application on uptake of N and P uptake by lentil plants was variable (Table 77, 81 and 85). Diammonium phosphate among fertilizers showed greatest beneficial impact on nutrient accumulation within lentil plants and consequently resulted in maximum accumulation of N in roots (27 mg/g) and shoots (57 mg/g) and P in roots (0.0.32 mg/g) and shoots (0.41 mg/g) which was calculated to be a significant increase of 42 and 36% (root and shoot N) and 39 and 32% (root and shoot P) respectively, in pot soils over control. *Pseudomonas putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* when used alone, significantly increased the N contents by 53 and 45% (root:shoot), 53 and 50% (root:shoot), 42 and 40% (root:shoot) and 63 and 55% (root:shoot) at 120 DAS compared to the control plants grown in pots. Similarly, under field trials, the *P. putida* and *B. pumilus*, *Azotobacter* and *Rhizobium* significantly ($P \leq 0.05$) enhanced the N contents in roots and shoots of lentil plants by 38 and 56%, 43 and 61%, 29 and 54% and, 52 and 72%, respectively relative to control plants. The P concentration in roots and shoots of lentil plants grown in pots following sole application of *P. putida* and *B. pumilus*, *Azotobacter* and *Rhizobium* significantly ($P \leq 0.05$) enhanced by 52 and 48%, 64 and 58%, 36 and 42% and, 64 and 55%, respectively, over control. While comparing the impact of all single microbial cultures on N and P contents in roots and shoots of lentil plants grown both in pots and field soils, it was observed that *B. pumilus* had the largest stimulatory effect on N and P concentration (Table 77, 81, 85). In a similar way, when effect of single application of microbial cultures was compared with those of urea and DAP used alone, it was found that microbial cultures in general had better effect on N and P concentration in both roots and shoots of pot and field grown lentil plants. *Rhizobium* inoculated plants in the presence of DAP (90 kg/ha) performed exceptionally well and dramatically enhanced the N contents in roots (84%) and shoots (69%) and P contents in roots (82%) and shoots (65%) in pot trials and 76, 82 (root and shoot N), 88 and 70% (root and shoot P) in field conditions, respectively over non inoculated and non- treated control plants. The co-culture of [*Rhizobium* and *B. pumilus*] markedly augmented the N concentration in roots and

shoots by 105 and 76% (pots) and 86, 95% (field) while P content in roots and shoots of pot grown plants was enhanced by 104 and 77% and in field grown plants it was 104 and 76% above pot/field control plants. Moreover, the impact of mixture of both urea (30 kg/ha) and DAP (90 kg/ha) on the measured parameters was statistically significant compared to other single treatment of urea or DAP or control plants in both pot and field trials. Of the two fertilizers, 90 kgDAP/ha in general, produced maximum positive effect on the measured parameters of either inoculated or un-inoculated lentil plants.

4.11.6 Seed yield and seed protein

Influence of microbial inoculations and fertilizer application on seed production and grain protein of lentil plants grown both in pot and field experiments and measured at harvest varied greatly among treatments (Table 77, 81 and 85). Fertilizers such as urea (30 kg/ha) and DAP (90 kg/ha) applied separately both in pots and field experiments even though differed in responses (Table 77) but it was statistically not significant ($P \leq 0.05$). Unlike fertilizers, the single inoculation of *P. putida*, *B. pumilus*, and *Azotobacter, Rhizobium*, significantly ($P \leq 0.05$) increased the seed yield by 62 (4.2 g/plant), 65 (4.3 g/plant), 54 (4 g/plant) and 104% (5.3 g/plant) relative to the control (2.6 g/plant). Moreover, in the presence of 30 kg urea/ha, *B. pumilus* had the maximum positive effect on seed formation in lentil and increased it significantly ($P \leq 0.05$) by 119% (5.7 g/plant) while *Rhizobium* in the presence of 90 kg DAP/ha augmented the seed yield by 123% (5.8 g/plant) compared to untreated and uninoculated plants grown in pots. Under field trials, *Rhizobium* in the presence of 90 kg DAP/ha gave maximum seed yield (132% increases) compared to control (2.5 g/plant). The composite cultures of [*B. pumilus* and *Rhizobium*] increased the grain yield maximally by 158% (pots) and 176% (field trials) which was followed by *P. putida* and *Rhizobium* inoculated lentil plants grown in pots (146%) and fields (172%) compared to control plants. Mixed application of recommended dose of urea and DAP increased the seed yield by 138% and 160% in pot and field conditions, respectively over control. Increase in protein content in lentil seeds following microbial cultures or fertilizer application (Table 77, 81, 85) was poor and ranged between 7 (30 kg urea/ha treatment alone) to 13% (*B. pumilus* + *Rhizobium*) in pot experiments. Similarly, the grain protein in seeds recovered from field grain lentil plants was statistically not different among treatments. The two way ANOVA in general revealed that the individual effect of inoculants (df=3), further application of fertilizer (df=1)

and interaction between inoculation and fertilizer (df=3) was significant ($P \leq 0.05$) for all measured parameters.

Table 14- Microbial diversity in different soil samples collected from experimental fields of Faculty of Agricultural Sciences

Soil samples	Microbial populations (colony forming unit/g soil)			Total microbial counts ($\times 10^7$)
	Bacteria ($\times 10^7$)	Fungi ($\times 10^5$)	Actinomycetes ($\times 10^4$)	
Non-rhizosphere (control)	2.43	0.8	1.2	**2.51**
Mentha	4.28	1.8	2.1	**4.30**
Chilli field	3.51	1.5	2.5	3.52
Cabbage field	3.42	1.4	2.8	3.43
Mustard field	3.85	1.7	3.2	3.87
Chickpea field	3.62	1.3	1.7	3.63
Pea field	3.71	1.2	1.6	3.72
Greengram field	4.21	1.4	1.9	**4.22**
Lentil field	3.94	1.1	2.1	3.96
Mean value	**3.71 $\times 10^7$)**	**1.43 ($\times 10^5$)**	**2.2 ($\times 10^4$)**	**3.83**

Table 15- Population of P-solubilizing and asymbiotic N-fixer in different soil samples collected from experimental fields of Faculty of Agricultural Sciences

Soil samples	Microbial populations (colony forming unit/g soil)			
	Phosphate solubilizer bacteria ($\times 10^5$) (A)	Phosphate solubilizing fungi ($\times 10^3$) (B)	PSM (A+B) ($\times 10^5$)	Asymbiotic nitrogen fixer ($\times 10^5$)
Mentha field	5.24	3.2	5.3	2.5
Cabbage field	4.36	4.2	4.4	2.1
Chilli field	4.76	3.8	4.8	2.6
Mustard field	3.95	4.8	4.0	1.9
Chickpea field	5.21	6.0	5.3	2.9
Pea field	6.32	6.8	**6.4**	3.2
Greengram field	7.17	6.4	**7.2**	3.1
Lentil field	4.98	7.1	5.1	2.7
Mean value	**5.24$\times 10^5$**	**5.20 $\times 10^3$**	**5.3$\times 10^5$**	**2.6 $\times 10^5$**

Table 16- Morphological and biochemical characteristics of N-fixers

Characterstics	N-fixer				
	Rhizobial group (N=150)				Azotobacter (N=50)
	RG	RP	RB	RV	AZ
Morphological					
Colony morphology	Transparent, circular and mucoid	Transparent, circular and mucoid	Transparent, circular and mucoid	Transparent, circular and mucoid	Serrated, muciliginous, Dark pigmented
Gram reaction	G-ve	G-ve	G-ve	G-ve	G-ve
Cell shape	Short rod	Short rod	Short rod	Short rod	Rod
Biochemical test					
Citrate	-	-	-	-	-
Indole	-	-	+	20	-
Methyl red	25	35	20	20	80
Nitrate reduction	30	30	20	20	85
Voges Proskauer	20	18	16	22	55
Catalase	30	30	20	20	100
Oxidase	-	-	-	-	-
Hydrolysis					
Starch	12	14	10	16	40
Gelatin	18	14	4	8	35
Carbohydrate utilization					
Glucose	26	24	16	16	90
Lactose	8	4	-	6	50
Mannitol	30	30	20	20	-
Sucrose	24	18	18	14	90
Arabinose	14	-	12	8	20
Xylose	-	-	-	-	40
Inositol	12	14	-	-	-
Mannose	-	-	8	-	40
Identification	*Mesorhizobium* sp.	*Rhizobium* sp.	*Bradyrhizobium* sp.	*Rhizobium* sp.	*Azotobacter* sp.

RG, RP, RB and RV indicate rhizobial species isolated from the nodules of chickpea, pea, greengram and lentil respectively. AZ indicate *Azotobacter* sp. isolated from rhizospheric soils. Values represents the percent of isolates expressing positive reaction to each biochemical test. "-"indicates a negative reaction

Table 17- Morphological and biochemical characteristics of P-solubilizers

Characterstics	P-solubilzer (N=50)				
	PSE (S1, S3)	PSE (S2,S4)	PSE (S2)	PSE (S2)	PSE (S3)
Morphological					
Colony morphology	Oily mucoid, smooth	Mucoid, smooth	Mucoid, serrate margin	Mucoid, smooth	Mucoid, smooth
Gram reaction	G-ve	G-ve	G+ve	G-ve	G-ve
Cell shape	Short rod	Short rod	Rod	Rod	Rod
Biochemical test					
Citrate	20	13	13	7	7
Indole	-	-	20	13	7
Methyl red	33	20	-	-	13
Nitrate reduction	20	13	7	13	7
Voges Proskauer	24	20	20	13	13
Catalase	24	20	20	13	13
Oxidase	24	-	-	7	13
Hydrolysis			+	+	+
Starch	13	7	13	-	-
Gelatin	16	7	7	13	7
Carbohydrate utilization			+	+	+
Glucose	24	20	20	13	13
Lactose	13	7	20	7	7
Mannitol	-	-	-	-	-
Sucrose	24	20	20	13	13
Arabinose	-	-	+	+	+
Xylose	13	7	13	7	7
Inositol	13	-	-	-	7
Mannose	13	7	13	-	-
	Pseudomonas sp.	*Achromobacter* sp.	*Bacillus* sp.	*Enterobacter* sp	*Stenotrophomonas* sp.

PSE indicate P-solubilizing bacteria isolated from rhizospheric soil; S1, S2, S3, S4 represents rhizospheric soil of mentha, chilli, cabbage and mustard respectively. Values represent the percent of isolates expressing positive reaction to each biochemical test. "-"indicates a negative reaction

Table 18- Plant growth promoting (PGP) and antifungal activity based typing of *Mesorhizobium* strains (N=15) isolated from chickpea nodules

PGP groups	Isolate designation	PGP activities								
		ACC deaminase	Phosphate solubilization	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	RG5	+	+	+	+	+	+	+	+	A,P,I,S,E,N,H,Af
II	RG4	+	-	+	+	+	+	+	-	A,I,S,E,N,H,Af
III	RG1, RG2, RG3, RG7, RG8	+	-	+	-	+	+	-	-	A,P,I,S,E,N,H,
IV	RG10, RG12,RG15	+	-	+	-	+	+	-	-	A,I,E,N,
V	RG6	-	+	+	-	+	+	-	-	P,I,E,N,
VI	RG9, RG11, RG13,RG14	-	-	+	-	+	+	-	-	I,E,N

In this and succeeding Tables, A= ACC deaminase; P= Phosphate solubilization; I= IAA; S= Siderophore;E= EPS (Exo-polysaccharides); N= Ammonia; H= HCN; Af= antifungal activity; CAS indicates Chrome azurol S agar; += positive activity and '-' indicates no reaction

Table 19- Plant growth promoting (PGP) and antifungal activity based typing of *Rhizobium* strains (N=15) isolated from pea nodules

PGP groups	Isolate designation	PGP activities								
		ACC deaminase	Phosphate solubilizatioı	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	RP2, RP6	+	+	+	+	+	+	+	+	A,P,I,S,E,N,H,Af
II	RP3, RP7, RP10	+	-	+	+	+	+	+	-	A,,I,S,E,N,H,
III	RP5, RP8	+	-	+	-	+	+	-	+	A,,I,E,N,Af
IV	RP14	-	-	+	-	+	+	-	-	I,E,N,H
V	RP1, RP4, RP9, RP11, RP12, RP13,RP15	-	-	+	-	+	+	-	-	I,E,N

Table 20- Plant growth promoting (PGP) and antifungal activity based typing of *Bradyrhizobium* strains (N=10) isolated from greengram nodules

PGP groups	Isolate designation	PGP activities								
		ACC deaminase	Phosphate solubilization	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	RB6	+	+	+	+	+	+	+	-	A,P,I,S,E,N,H
II	RB10	-	-	+	+	+	+	+	+	I,S,E,N,H,Af
III	RB1, RB2,RB7	-	-	+	+	+	+	+	-	I,S,E,N,H
V	RB3	+	-	+	-	+	+	-	+	A,I,E,N,Af
V	RB4, RB5, RB8	-	-	+	-	+	+	-	-	I,E,N
VI	RB9	-	-	-	-	+	+	+	-	E,N,H

Table 21- Plant growth promoting (PGP) and antifungal activity based typing of *Rhizobium* strains (N=10) isolated from lentil nodules

PGP groups	Isolate designation	PGP activities								
		ACC *deaminase*	Phosphate *solubilization*	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	RV6, RV8	+	+	+	+	+	+	+	-	A,P,I,S,E,N,H
II	RV3	+	-	+	+	+	+	+	-	A,,I,S,E,N,Af
III	RV2	+	-	+	-	+	+	-	-	A,I,S,E,N,H
IV	RV1	-	-	+	+	+	+	-	+	I,S,E,N,Af
V	RV4, RV5, RV7, RV8	-	-	+	-	+	+		-	I,E,N
VI	RV10	-	-	-	-	+	+	+	-	E,H,N

Table 22- Plant growth promoting (PGP) and antifungal activity based typing of *Azotobacter* strains (N=20) isolated from rhizospheric soil

PGP groups	Isolate designation	PGP activities							
		Phosphate solubilization	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	AZ1, AZ13, AZ19	+	+	+	+	+	+	+	P,I,S,E,N,H,Af
II	AZ2, AZ5, AZ7,AZ10, AZ11, AZ17 ,AZ18	-	+	+	+	+	+	+	P/S,I,E,N,H,Af
III	AZ4, AZ6	+	+	-	+	+	-	+	P,I,E,N,Af
IV	AZ2, AZ3, AZ12, AZ14, AZ20	+	+	-	+	+	-/+	-/+	P,I,E,N
V	AZ9, AZ15, AZ16	-	+	-	+	+	-	-	I,E,N

Table 23- Plant growth promoting (PGP) and antifungal activity based typing of phosphate solubilizers (N=30) isolated from rhizospheric soils

PGP groups	Isolate designation	PGP activities								
		ACC deaminase	Phosphate solubilization	IAA	Siderophore on CAS agar	EPS	NH_3	HCN	Antifungal activity	Activity profile
I	PSE3, PSE5, PSE9, PSE15, PSE18, PSE24, PSE28, PSE23	+	+	+	+	+	+	+	+	A,P,I,S,E,N,H,Af
II	PSE19	+	+	+	+	+	+	-	+	A,P,I,S,E,N,Af
III	PSE6, PSE8, PSE12, PSE27 PSE16, PSE20, PSE22, PSE26	-/+	+	+	-/+	+	+	-/+	-	P,I,S,E,N,H
IV	PSE10, PSE11, PSE13 PSE14, PSE21	-/+	+	+	+/-	+/-	+	-/+	-/+	P,I,S,E,N
V	PSE1, PSE2, PSE7, PSE25	-/+	+	+	-/+	-	+	-/+	-	P,I,H,N
\VI	PSE4, PSE17	-	+	+		-	+	-	-	P,I,N

Table 24- Quantitative assay of plant growth promoting (PGP) activity of *Mesorhizobium* strains (N=15) isolated from chickpea nodules

Bacterial strain	ACC deaminase activity (µmol α-ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
RG1	113±12	-	-	-	-	25±3	64±6	10±1.3	13±1.4	26±2	20±2
RG2	201±19	-	-	-	-	21±4	58±5	11±1.2	14±1.6	32±3	23±2
RG3	152±15	-	-	-	-	18±3	32±4	12±1.2	12±1.3	25±3	24±3
RG4	258±27	-	-	-	-	32±4	75±9	11±1.3	15±1.7	29±4	32±3
RG5	232±23	1.6±0.4	57±6	75±9	5.8±0.5	28±3	64±7	12±1.4	13±1.4	28±3	26±2
RG6	-	1.4±0.3	42±4	64±11	6.1±0.6	14±2	34±6	-	-	-	25±2
RG7	158±14	-	-	-	-	18±3	42±5	11±1.2	15±1.5	34±4	29±3
RG8	115±12	-		-	-	22±4	53±7	12±1.5	16±1.3	33±4	26±2
RG9	-	-		-	-	28±5	65±8	-	-	-	31±3
RG10	191±16	-		-	-	29±3	64±5	-	-	-	19±2
RG11	-	-		-	-	19±2	43±6	-	-	-	21±2
RG12	182±20	-		-	-	21±3	54±6	-	-	-	26±2
RG13	-	-		-	-	15±3	37±4	-	-	-	29±3
RG14	-	-		-	-	14±2	32±5	-	-	-	23±2
RG15	165±15	-		-	-	25±3	63±9	-	-	-	24±2

In this and succeding tables SI indicates solubilization index= [(zone size including colony diameter)/ colony diameter]; SE= solubilizing efficiency [(zone of solubilization/colony diameter)×100][T-Trypptophan concentration (µg/ml); CAS- Chrome Azurol S agar; SA- Salicylic acid; DHBA- 2,3 Dihydroxy benzoic acid; EPS- Exopolysaccharides

Values in this and succeeding tables indicate the mean ± S.D. of three independent replicates

Table 25- Quantitative assay of plant growth promoting (PGP) activity of *Rhizobium* strains (N=15) isolated from pea nodules

Bacterial strain	ACC deaminase activity (µmol α-ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
RP1	-	-	-	-	-	18±3	42±5	-	-		23±2
RP2	238±11	1.8±0.3	83±7	81±22	5.8±0.3	26±4	65±7	11±1.3	12±1.5	24±2.1	28±3
RP3	155±8	-	-	-	-	19±3	41±5	13±1.2	15±1.8	19±1.8	25±3
RP4	-	-	-	-	-	24±5	72±8	-	-	-	19±2
RP5	156±9	-	-	-	-	20±4	53±6	-	-	-	23±3
RP6	211±14	1.5±0.3	50±6	65±12	6.1±0.3	19±3	47±5	12±1.4	16±1.6	28±2.6	32±4
RP7	182±7	-	-	-	-	25±5	67±8	11±1.1	14±1.3	27±2.4	35±4
RP8	164±8	-	-	-	-	28±5	75±8	-	-	-	21±3
RP9	-	-	-	-	-	32±5	73±7	-	-	-	19±3
RP10	132±6	-	-	-	-	18±4	45±5	10±1.1	13±1.2	31±2.5	24±3
RP11	-	-	-	-	-	16±3	41±6	-	-	-	18±2
RP12	-	-	-	-	-	17±3	56±5	-	-	-	28±4
RP13	-	-	-	-	-	22±5	62±7	-	-	-	15±3
RP14	-	-	-	-	-	26±5	68±7	-	-	-	17±2
RP15	=	-	-	-	-	13±3	41±5	-	-	-	27±4

Table 26- Quantitative assay of plant growth promoting (PGP) activity of *Bradyhizobium* strains (N=10) isolated from greengram nodules

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
RB1	-	-	-	-	-	32±4	79±9	12±1.3	15±1.6	31±2.5	21±3
RB2	-	-	-	-	-	35±3	91±8	11±1.2	16±1.8	35±2.8	15±2
RB3	185±12	-	-	-	-	38±5	85±10	-	-	-	17±2
RB4	-	-	-	-	-	41±5	95±11	-	-	-	23±3
RB5	-	-	-	-	-	26±3	69±7	-	-	-	26±4
RB6	211±15	2.2±0.4	117±8	148±24	5.9±0.4	36±4	74±8	12±1.5	14±1.6	25±2.1	15±3
RB7	-	-	-	-	-	29±3	68±7	10±1.2	15±1.4	31±2.7	23±3
RB8	-	-	-	-	-	21±3	54±6	-	-	-	18±2
RB9	-	1.4±0.4	42±7	111±13	5.8±0.4	-	-	-	-	-	14±2
RB10	-	-	-	-	-	32±4	81±9	11±1.4	14±1.6	26±2.4	28±4

Table 27- Quantitative assay of plant growth promoting (PGP) activity of *Rhizobium* strains (N=10) isolated from lentil nodules

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
RV1	225±14	-	-	-	-	32±3	69±7	11±1.2	14±1.5	31±2.9	31±3
RV2	-	-	-	-	-	18±3	42±6	10±1.2	15±1.6	28±2.7	23±3
RV3	248±16	-	-	-	-	37±4	89±9	12±1.3	13±1.2	24±2.8	17±2
RV4	-	-	-	-	-	25±3	58±6	-	-	-	26±3
RV5	-	-	-	-	-	29±4	67±8	-	-	-	15±2
RV6	185±13	1.5±.03	50±6	123±14	5.8±.03	38±4	92±8	13±1.4	16±1.5	29±3.1	28±3
RV7	-	-	-	-	-	26±2	63±7	-	-	-	21±3
RV8	-	-	-	-	-	31±3	78±9	-	-	-	26±3
RV9	201±16	1.8±.04	83±.7	131±12	5.7±.04	28±3	65±7	12±1.3	14±1.5	32±2.5	25±3
RV10	-	-	-	-	-	-	-	-	-	-	24±3

Table 28- Quantitative assay of plant growth promoting (PGP) activity of *Azotobacter* strains (N=20) isolated from various rhizospheric soil

Bacterial strain	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
	S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA µg/ml)	SA (µg/ml)	
AZ1	2.0±0.4	100±6	165±12	5.9±0.4	32±3	74±8	12±1.4	16±1.5	31±2.3	31±3
AZ 2	-	-	-		28±3	65±7	10±1.3	15±1.7	28±2.5	42±3
AZ 3	-	-	-		25±3	53±6	-	-	-	45±4
AZ 4	2.4±0.5	140±9	212±17	5.6±0.3	42±4	89±8	-	-	-	37±5
AZ 5	2.5±0.5	150±8	188±8	5.3±0.4	36±4	78±8	13±1.4	17±1.4	34±2.8	39±3
AZ 6	-	-	-		28±3	65±7	12±1.2	15±1.6	31±2.6	43±4
AZ 7	1.7±0.4	67±7	145±7	5.6±0.5	37±4	77±8	11±1.5	15±1.7	28±2.2	31±3
AZ 8	1.5±0.3	50±8	122±8	5.6±0.4	41±4	93±8	-	-	-	21±3
AZ 9	-	-	-		34±4	75±7	-		-	26±4
AZ 10	2.5±0.5	150±9	182±9	5.2±0.3	38±4	85±6	12±1.3	17±1.8	29±2.8	29±3
AZ 11	-	-	-		27±4	68±7	13±1.1	18±1.9	31±2.7	41±4
AZ 12	1.6±0.4	60±7	117±7	5.9±0.4	37±5	78±8	-	-	-	19±2
AZ 13	1.8±0.3	80±7	122±9	5.8±0.5	35±3	81±7	12±1.3	16±1.4	35±2.5	28±3
AZ 14	-	-	-		19±3	46±5	-		-	37±4
AZ 15	-	-	-		23±3	59±6	-		-	32±3
AZ16	-	-	-		29±4	75±6	-		-	25±3
AZ17	2.3±0.5	133±10	188±10	5.5±0.4	31±4	69±8	13±1.2	18±1.5	31±2.9	43±4
AZ18	-	-	-		24±3	65±6	11±1.2	15±1.7	31±2.5	21±3
AZ19	2.6±0.4	160±11	215±11	5.2±0.4	42±4	96±9	12±1.1	15±1.6	28±2.4	19±2
AZ20	1.5±0.2	50±4	87±8	5.8±0.4	37±4	91±8	-	-	-	39±3

Table 29- Quantitative assay of plant growth promoting (PGP) activity of P- solubilizers (N=8) isolated from mentha rhizospheric soil

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
PSE1	-	1.6±0.3	60±8	145±11	5.7±0.3	15±2	38±3	-	-	-	-
PSE2	345±15	2.2±0.4	120±11	180±13	5.1±0.4	18±2	43±4	-	-	-	-
PSE3	652±25	4.3±0.5	219±15	319±24	5.1±0.5	14±1	29±3	12±1.7	15±1.7	37±3	25±2
PSE4	-	1.6±0.3	60±9	165±12	5.5±0.3	11±1	32±3	-	-	-	-
PSE5	545±23	3.8±0.4	226±15	286±17	5.2±0.4	17±2	39±4	11±1.6	11±1.1	28±2	19±2
PSE6	-	2.2±0.5	120±6	211±16	5.5±0.5	17±2	65±3	13±1.3	14±1.2	32±3	22±2
PSE7	-	1.8±0.3	80±9	177±15	5.8±0.3	23±2	62±4	-	-	-	-
PSE8	-	2.4±0.3	140±12	214±18	5.6±0.3	25±3	71±5	12±1.5	16±1.4	29±3	-

Table 30- Quantitative assay of plant growth promoting (PGP) activity of P- solubilizers (N=10) isolated from chilli rhizospheric soil

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
PSE9	227±12	1.6±0.3	60±5	163±12	5.6±0.3	14±1	34±3	12±1.3	18±1.6	34±2	23±3
PSE 10	-	2.2±0.4	120±8	190±13	5.1±0.4	18±2	42±3	10±1.1	17±1.4	38±3	21±2
PSE 11	458±18	2.4±0.3	140±11	211±15	5.3±0.3	17±2	41±4	-	-	-	-
PSE 12	238±13	2.8±0.5	180±14	235±14	5.7±0.5	15±1	36±3	12±1.3	16±1.3	32±3	-
PSE 13	-	1.8±0.3	80±6	195±12	5.8±0.3	21±2	57±4	-	-	-	16±2
PSE 14	488±21	3.2±0.5	220±11	276±16	5.1±0.5	16±2	47±4	-	-	-	-
PSE 15	565±23	2.8±0.4	80±8	256±15	5.4±0.4	19±2	59±4	13±1.2	15±1.4	37±3	22±2
PSE 16	-	1.5±0.4	50±4	154±11	5.5±0.4	22±3	63±6	-	-	-	14±1
PSE 17	-	2.7±0.3	267±16	267±16	5.7±0.3	24±2	58±4	-	-	-	-
PSE 18	345±14	3.4±0.4	240±13	265±13	5.4±0.4	14±1	45±4	11±1.2	14±1.6	31±3	18±2

Table 31- Quantitative assay of plant growth promoting (PGP) activity of P- solubilizers (N=7) isolated from cabbage rhizospheric soil

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
PSE19	335±21	2.2±0.3	117±11	186±12	5.1±0.3	23±2	59±3	13±1.2	18±1.5	31±2	23±2
PSE 20	446±23	2.3±0.2	80±7	156±11	4.8±0.2	13±2	43±4	-	-	-	13±2
PSE 21	-	3.4±0.4	240±11	231±13	4.4±0.4	17±2	76±6	12±1.1	19±1.7	34±3	-
PSE 22	278±18	2.8±0.3	180±7	189±12	5.2±0.3	18±2	54±4	10±1.0	21±2.0	38±4	18±2
PSE 23	232±17	2.5±0.2	150±9	211±11	5.1±0.2	16±2	65±4	-	-	-	14±1
PSE 24	578±25	3.2±0.3	220±8	235±12	4.7±0.3	27±2	62±5	13±1.2	18±1.5	29±2	21±2
PSE 25	-	2.3±0.3	133±9	186±10	5.3±0.3	31±3	79±4	12±1.1	17±1.4	32±3	-

Table 32 - Quantitative assay of plant growth promoting (PGP) activity of P- solubilizers (N=5) isolated from mustard rhizospheric soil

Bacterial strain	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
		S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
PSE26	467±18	1.6±0.2	60±7	156±12	5.6±0.2	24±3	54±4	13±1.2	17±1.7	32±3	17±2
PSE 27	-	2.8±0.3	180±10	211±14	5.7±0.3	18±2	48±4	12±1.1	18±2	34±3	27±3
PSE 28	548±21	3.6±0.4	260±11	321±18	4.6±0.4	17±2	57±5	13±1.4	21±2	37±4	21±2
PSE 29	398±16	2.5±0.3	150±13	198±13	5.5±0.3	25±3	65±6	-	-	-	
PSE 30	-	1.8±0.2	125±8	145±11	5.8±0.2	13±2	39±4	-	-	-	25±3

Table 33-Antifungal activity of rhizobia and *Azotobacter*

Bacterial group	Isolate designation	Zone of inhibition (mm)		
		Rhizoctonia sp.	*Penicillium* sp.	*Alternaria* sp.
Mesorhizobium	RG4	24.2±1.1	21.1±0.9	23.6±1.2
	RG5	21.3±1.2	18.4±0.8	22.6±1.3
Rhizobium (Pea)	RP5	27.3±1.3	26.4±0.9	26.9±0.7
	RP6	24.5±1.2	23.6±1.2	25.1±0.6
	RP8	31.8±1.5	29.1±1.4	29.8±0.8
Bradyrhizobium	RB3	31.1±1.7	27.6±1.2	28.6±1.1
	RB10	27.6±1.5	24.1±1.3	25.3±1.2
Rhizobium (lentil)	RV3	28.6±1.1	26.4±1.2	27.6±1.2
	RV7	31.2±1.3	27.5±1.5	24.1±1.5
	RV9	22.9±1.2	21.1±1.6	22.8±1.1
Azotobacter	AZ1	26.3±1.5	21.8±0.9	24.4±1.5
	AZ2	21.5±1.8	19.1±1.3	20.2±1.1
	AZ3	17.3±1.2	15.2±1.5	16.7±1.1
	AZ4	14.7±1.4	14.6±0.9	13.6±1.6
	AZ5	18.1±1.9	18.1±0.8	15.8±1.2
	AZ10	25.8±1.6	23.7±1.4	19.6±1.5
	AZ11	28.9±1.8	26.2±1.6	24.6±1.6
	AZ13	32.3±1.2	27.7±1.4	27.9±1.3
	AZ17	28.5±1.7	24.9±1.1	24.1±1.7
	AZ18	26.1±1.7	25.1±1.5	23.2±1.7
	AZ19	20.5±1.8	21.2±1.2	25.1±1.5

Value indicates mean± standard deviation of three independent replicates

Table 34- Antifungal activity of isolated P- solubilizers

Isolate designation	Zone of inhibition (mm)		
	Rhizoctonia sp.	*Penicillium* sp.	*Alternaria* sp.
PSE3	19.3±1.3	13.4±0.9	15.6±1.1
PSE5	23.1±1.4	18.1±1.2	21.9±1.2
PSE7	24.2±1.1	21.4±1.5	22.1±1.4
PSE8	14.9±1.2	12.5±0.9	13.2±1.1
PSE9	27.2±1.4	24.1±1.5	25.2±1.2
PSE12	14.3±0.9	13.7±0.8	14.2±1.3
PSE14	18.5±1.6	17.4±1.6	18.1±1.7
PSE15	23.1±1.6	22.2±1.7	22.7±1.2
PSE16	16.8±1.2	15.7±1.1	15.1±1.1
PSE18	19.3±1.3	18.1±2.3	18.3±1.5
PSE19	28.1±1.6	26.8±1.2	25.1±1.2
PSE20	21.6±1.4	20.4±1.6	20.4±1.4
PSE23	23.2±1.2	22.1±1.1	22.7±1.3
PSE24	32.4±1.7	28.2±1.2	29.2±1.6
PSE28	28.6±1.2	26.9±0.7	25.1±1.2
PSE29	17.2±1.5	16.1±1.4	16.6±1.6

Value indicates mean± standard deviation of three independent replicates

Table 35- Antibiotic resistance/ sensitivity profile of most promising PGPR strain

Bacterial strains	Zone of inhibition (mm)																	Resistance pattern	R (%)	S (%)
	Am	Cf	Cx	C	Do	E	G	K	M	NA	Nf	Nx	Nv	P	Pb	R	T			
RG5	R	32	R	25	32	21	25	21	R	R	R	18	R	R	R	19	21	Am, Cx, M, NA, Nf, Nv, P, Pb	47.0	53.0
RP2	19	31	R	31	18	23	27	23	R	R	R	21	R	R	R	21	20	Cx, M, NA, Nf, Nv, P, Pb	41.2	58.8
RB6	R	35	R	28	19	20	R	24	R	R	R	20	R	R	R	R	22	Am, Cx, G, M, NA, Nf, Nv, P, Pb, R	58.8	41.2
RV9	21	34	R	23	24	24	19	24	R	19	R	22	R	R	R	23	23	Cx, M, NA, Nf, Nv, P, Pb	35.3	64.7
AZ19	17	28	R	32	R	R	R	27	R	R	R	19	R	R	R	R	19	Cx, Do, E, G, M, NA, Nf, Nv, P, Pb, R	64.7	35.3
PSE3	19	36	R	35	17	R	25	25	R	R	R	23	R	R	R	R	18	Cx, E, M, NA, Nf, Nv, P, Pb, R	52.9	47.1
PSE5	21	31	R	21	23	32	32	21	R	R	R	19	R	R	R	24	21	Cx, M, NA, Nf, Nv, P, Pb	41.2	58.9
PSE9	R	24	R	29	28	28	R	22	R	R	R	21	R	R	R	19	18	Am, Cx, G, M, NA, Nf, Nv, P, Pb	52.9	41.1
PSE15	23	32	R	25	32	18	28	20	R	R	R	22	R	R	R	21	R	Cx, M, NA, Nf, Nv, P, Pb, R	47.0	63.0
PSE18	18	36	R	24	R	19	23	23	R	R	R	19	R	R	R	R	21	Cx, Do, M, NA, Nf, Nv, P, Pb, R	52.9	47.1
PSE19	R	28	R	19	R	R	R	19	R	R	R	18	R	R	R	23	R	Am,Cx,Do,E, G, M, NA, Nf, Nv, P, Pb,R,T	70.6	29.4
PSE24	17	31	R	31	34	R	R	21	R	R	R	R	R	R	R	R	R	Cx,E, G, M, NA, Nx,Nf, Nv, P, Pb,R,T	70.6	29.4
PSE28	19	32	R	23	23	27	24	23	R	R	R	21	R	R	R	19	19	Cx, M, NA, Nf, Nv, P, Pb	41.2	58.9

In this table values<12 mm were considered resistant as recommended by the disc manufacturer

Table 36- Molecular identification of bacterial strains by 16S rRNA gene sequencing

S.No.	Original designation	Designation for submission to NCBI	Bacterial strain	GenBank accession No.	Nucleotide sequence (bp)	Data base	Source
1	PSE3	PSE3	*Pseudomonas putida*	HM236047	933	NCBI	Mentha
2	PSE5	PSE5	*Pseudomonas putida*	HM236048	937	NCBI	Mentha
3	PSE9	ES1	*Achromobacter*	JX483710	996	NCBI	Chilli
4	PSE15	ES2	*Enterobacter*	JX965901	1017	NCBI	Chilli
5	PSE18	ES3	*Bacillus pumilus*	JX965902	984	NCBI	Chilli
6	PSE19	ES4	*Pseudoxanthomonas*	JX965903	1640	NCBI	Cabbage
7	PSE24	ES5	*Stenotrophomonas*	JX965904	783	NCBI	Cabbage
8	PSE28	ES6	*Achromobacter*	JX965905	982	NCBI	Mustard

Table 37- Most promising bacterial strains showing greatest plant growth promoting activity

Bacterial strain	Strain name	ACC deaminase activity (µmol α- ketobutyrate/mg protein/h)	P-solubilization				IAA (µg/ml)		Siderophore			EPS (µg/ml)
			S.I.	S.E.	Liquid (µg/ml)	Change in pH	0 T	100 T	Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)	
RG5	*M. ciceri*	232±23	1.6±0.4	57±5	75±9	5.8±0.5	28±3	64±7	12±1.4	13±1.4	28±3	26±2
RP2	*R. leguminosarum*	238±11	1.8±0.3	83±7	81±22	5.8±0.3	26±4	65±7	11±1.3	12±1.5	24±2.1	28±3
RB6	*Bradyrhizobium*	211±15	2.2±0.4	117±8	148±24	5.9±0.4	36±4	74±8	12±1.5	14±1.6	25±2.1	15±3
RV9	*Rhizobium*	185±13	1.8±.04	83±.7	131±12	5.7±.04	38±4	92±8	13±1.4	16±1.5	29±3.1	28±3
AZ19	*Azotobacter*	N.A.	2.6±0.4	160±11	215±11	5.2±0.4	42±4	96±9	12±1.1	15±1.6	28±2.4	19±2
PSE3	*P. putida*	652±25	4.3±0.5	219±15	319±24	5.1±0.5	14±1	29±3	12±1.7	15±1.7	37±3	25±2
PSE5	*P. putida*	545±23	3.8±0.4	226±15	286±17	5.2±0.4	17±2	39±4	11±1.6	11±1.1	28±2	19±2
ES1	*Achromobacter*	227±12	1.6±0.3	60±5	163±12	5.6±0.3	14±1	34±3	12±1.3	18±1.6	34±2	23±3
ES2	*Enterobacter*	565±23	2.8±0.4	80±8	256±15	5.4±0.4	19±2	59±4	13±1.2	15±1.4	37±3	22±2
ES3	*B. pumilus*	345±14	3.4±0.4	240±13	265±13	5.4±0.4	14±1	45±4	11±1.2	14±1.6	31±3	18±2
ES4	*Pseudoxanthomonas*	335±21	2.2±0.3	117±11	186±12	5.1±0.3	23±2	59±3	13±1.2	18±1.5	31±2	23±2
ES5	*Stenotrophomonas*	578±25	3.1±0.3	3.2±0.3	220±8	235±12	4.7±0.3	62±5	13±1.2	18±1.5	29±2	21±2
ES6	*Achromobacter*	548±21	3.6±0.4	260±11	321±18	4.6±0.4	17±2	57±5	13±1.4	21±2	37±4	21±2

'R' indicates rhizobial group 'AZ' represents *Azotobacter* and 'PS' indicates phosphate solubilizing bacteria; NA-not analyzed

Table 38-Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on growth of chickpea plant

Treatments	Length/plant (cm)							
	Pot trials				Field trials			
	Root		Shoot		Root		Shoot	
	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control	21.1	23.2	24.1	26.2	24.2	26.2	27.4	28.2
Urea (30kg/ha)	24.3	26.5	29.2	31.4	28.4	31.5	32.3	34.4
DAP (80 kg/ha)	25.5	24.8	31.3	32.3	29.5	30.3	34.2	35.5
P. putrid	28.6	29.6	34.2	36.3	32.2	33.7	33.4	36.3
M. ciceri	31.3	31.3	33.2	37.5	31.3	34.1	34.5	36.2
P. putida +Urea	30.8	31.2	35.6	36.4	32.2	35.2	36.4	39.5
M. ciceri +DAP	31.2	32.3	36.3	38.3	33.3	35.6	37.6	39.2
P. putida+ *M. ciceri*	33.5	35.5	38.5	41.2	35.1	37.4	39.3	40.3
Urea+ DAP	31.2	32.4	37.3	39.5	34.2	36.5	38.2	39.2
LSD	2.72	2.93	3.14	3.33	3.43	3.22	3.63	4.13
F value Inoculation	78.5*	88.1*	76.5*	82.5*	71.5*	62.2*	95.4*	58.9*
Fertilizer	3.2*	2.6*	3.2*	2.2*	3.5*	2.8*	4.5*	3.2*
Interaction	2.3*	1.3*	1.4*	1.1*	2.1*	1.3*	1.6*	2.5*

In this and succeeding tables, values are mean of three replicates where each replicate constituted three plants/pot or plot, [a]Control receiving neither fertilizers nor inoculant *P. putida* strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN *M. ciceri* strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 39- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on dry biomass of plant organs for chickpea plant

Treatments		Dry biomass (g/plant)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control		0.93	0.98	2.11	2.92	1.12	1.18	2.20	3.00
Urea (30kg/ha)		1.23	1.31	2.35	3.45	1.34	1.38	2.45	3.62
DAP (80 kg/ha)		1.25	1.29	2.41	3.51	1.38	1.41	2.51	3.67
P. putrid		1.31	1.36	2.46	3.55	1.41	1.43	2.55	3.78
M. ciceri		1.29	1.39	2.48	3.51	1.42	1.45	2.54	3.83
P. putida +Urea		1.39	1.48	2.56	3.64	1.44	1.46	2.82	3.96
M. ciceri +DAP		1.37	1.46	2.59	3.71	1.43	1.47	2.86	3.98
P. putida+ *M. ciceri*		1.42	1.51	2.63	3.93	1.49	1.52	3.21	4.11
Urea+ DAP		1.41	1.47	2.61	3.81	1.45	1.47	3.12	4.01
LSD		0.25	0.29	0.31	0.41	0.17	0.18	0.33	0.44
F value	Inoculation	71.1*	78.2*	96.5*	82.1*	81.5*	62.2*	91.3*	78.9*
	Fertilizer	3.1*	2.8*	3.5*	2.8*	3.6*	2.8*	4.1*	4.2*
	Interaction	2.2*	1.1*	2.4*	1.7*	1.4*	1.3*	1.2*	1.5*

P. putida strain PSE3 produced ACC deaminase-625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced ACC deaminase-232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN

Table 40- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on photosynthetic and symbiotic attributes of chickpea plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		1.54	23	190	0.15	1.45	27	195	0.17
Urea (30kg/ha)		1.73	31	312	0.17	1.76	31	342	0.23
DAP (80 kg/ha)		1.71	33	323	0.18	1.78	32	345	0.25
P. putrid		1.73	45	415	0.24	1.79	41	415	0.31
M. ciceri		1.69	52	450	0.28	1.82	54	464	0.36
P. putida +Urea		1.78	46	445	0.25	1.87	46	424	0.31
M. ciceri +DAP		1.77	45	442	0.29	1.89	51	443	0.32
P. putida+ *M. ciceri*		1.81	59	489	0.34	1.94	55	492	0.39
Urea+ DAP		1.81	51	451	0.31	1.90	45	436	0.33
LSD		0.24	3.1	23.2	0.11	0.24	4.3	26.1	0.11
F value	Inoculation	78.5*	88.1*	76.5*	82.5*	71.5*	62.2*	95.4*	58.9*
	Fertilizer	3.2*	2.6*	3.2*	2.2*	3.5*	2.8*	4.5*	3.2*
	Interaction	2.3*	1.3*	1.4*	1.1*	2.1*	1.3*	1.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of chickpea growth

P. putida strain PSE3 produces 625 µmol α- ketobutyrate/mg protein/h ACC deaminase, amount of P-solubilization- 319 µg/ml in liquid medium; IAA produces 14 and 29 µg/ml at 0T and 100T respectively; synthesize 15 and 37 µg/ml DHBA and SA respectively; 25 µg/ml EPS produces; positive for ammonia and HCN production
M. ciceri strain RG5 produces 232 µmol α- ketobutyrate/mg protein/h ACC deaminase, amount of P-solubilization- 75 µg/ml in liquid medium; IAA produces 28 and 64 µg/ml at 0T and 100T respectively; synthesize 13 and 28 µg/ml DHBA and SA respectively; 26 µg/ml EPS produces; positive for ammonia and HCN production

Table 41- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on nutrient uptake, seed yield and seed quality of chickpea plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		15	26	0.16	0.24	2.8	242	18	31	0.18	0.27	2.6	244
Urea (30kg/ha)		25	31	0.21	0.29	3.8	254	27	36	0.23	0.32	3.8	256
DAP (80 kg/ha)		24	29	0.24	0.31	4.1	257	29	33	0.29	0.38	4.2	258
P. putrid		28	34	0.27	0.32	4.4	256	32	39	0.34	0.41	4.6	259
M. ciceri		29	37	0.26	0.29	4.3	261	38	42	0.29	0.36	4.7	262
P. putida +Urea		34	45	0.31	0.38	5.1	263	41	48	0.32	0.41	5.3	266
M. ciceri +DAP		38	44	0.34	0.41	5.3	262	39	46	0.41	0.47	5.5	268
P. putida+ *M. ciceri*		42	48	0.39	0.43	5.9	265	45	53	0.48	0.53	6.2	270
Urea+ DAP		41	45	0.37	0.39	5.1	264	42	46	0.46	0.51	5.5	266
LSD		2.5	3.1	0.13	0.14	1.2	11.3	2.8	3.2	0.15	0.16	1.3	11.3
F value	Inoculation	63.5*	81.1*	86.5*	79.5*	112.5*	82.2*	85.4*	123.9*	92.5*	128.5*	116.5*	72.2*
	Fertilizer	2.5*	3.6*	3.4*	3.2*	3.5*	3.8*	3.5*	3.6*	2.8*	3.8*	4.5*	3.5*
	Interaction	2.1*	2.3*	1.8*	2.1*	1.1*	2.3*	2.6*	1.5*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 135 DAS;

P. putida strain PSE3 produces 625 µmol α- ketobutyrate/mg protein/h ACC deaminase, amount of P-solubilization- 319 µg/ml in liquid medium; IAA produces 14 and 29 µg/ml at 0T and 100T respectively; synthesize 15 and 37 µg/ml DHBA and SA respectively; 25 µg/ml EPS produces; positive for ammonia and HCN production'

M. ciceri strain RG5 produces 232 µmol α- ketobutyrate/mg protein/h ACC deaminase, amount of P-solubilization- 75 µg/ml in liquid medium; IAA produces 28 and 64 µg/ml at 0T and 100T respectively; synthesize 13 and 28 µg/ml DHBA and SA respectively; 26 µg/ml EPS produces; positive for ammonia and HCN production

Table 42-Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on growth of chickpea plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control		20.8	23.1	24	26	24	26	27	28
Urea (30kg/ha)		24.1	26.2	29	31	28	31	32	34
DAP (80 kg/ha)		24.8	23.8	31	32	29	30	34	35
B. pumilus		26.7	30.2	32	35	31	32	34	36
M. ciceri		31.2	31.1	33	37	31	34	34	36
B. pumilus +Urea		30.1	29.6	34	36	31	34	37	38
M. ciceri +DAP		30.9	31.8	36	38	33	35	37	39
B. pumilus + *M. ciceri*		33.8	35.8	37	42	36	38	37	41
Urea+ DAP		31.2	32.1	37	39	34	36	38	39
LSD		2.62	3.12	3.2	3.0	3.5	3.1	3.7	4.0
F value	Inoculation	78.5*	88.1*	76.5*	82.5*	71.5*	62.2*	95.4*	58.9*
	Fertilizer	3.2*	2.6*	3.2*	2.2*	3.5*	2.8*	4.5*	3.2*
	Interaction	2.3*	1.3*	1.4*	1.1*	2.1*	1.3*	1.6*	2.5*

[a]Control receiving neither fertilizers nor inoculant

P B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 43- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on dry biomass of plant organs for chickpea plant

Treatments		Dry biomass g/plant							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control		0.93	0.98	2.11	2.92	1.12	1.18	2.20	3.00
Urea (30kg/ha)		1.23	1.31	2.35	3.45	1.34	1.38	2.45	3.62
DAP (80 kg/ha)		1.25	1.29	2.41	3.51	1.38	1.41	2.51	3.67
B. pumilus		1.35	1.39	2.51	3.59	1.44	1.48	2.61	3.83
M. ciceri		1.29	1.39	2.48	3.51	1.42	1.45	2.54	3.83
B. pumilus +Urea		1.38	1.45	2.58	3.71	1.48	1.53	2.88	3.99
M. ciceri +DAP		1.37	1.46	2.59	3.71	1.43	1.47	2.86	3.98
B. pumilus + *M. ciceri*		1.44	1.55	2.72	3.99	1.56	1.59	3.27	4.18
Urea+ DAP		1.41	1.47	2.61	3.81	1.45	1.47	3.12	4.01
LSD		0.23	0.31	0.33	0.39	0.19	0.16	0.35	0.45
F value	Inoculation	149.5*	98.1*	79.5*	92.4*	79.4*	68.2*	94.4*	53.9*
	Fertilizer	3.2*	2.1*	3.5*	2.1*	3.3*	2.4*	4.2*	4.2*
	Interaction	2.1*	1.3*	1.2*	1.7*	2.0*	1.1*	1.3*	2.1*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 44- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on photosynthetic and symbiotic attributes of chickpea plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		1.54	23	190	0.15	1.45	27	195	0.17
Urea (30kg/ha)		1.73	31	312	0.17	1.76	31	342	0.23
DAP (80 kg/ha)		1.71	33	323	0.18	1.78	32	345	0.25
B. pumilus		1.74	48	435	0.24	1.78	43	396	0.33
M. ciceri		1.69	52	450	0.28	1.82	54	464	0.36
B. pumilus +Urea		1.77	45	424	0.26	1.89	39	387	0.32
M. ciceri +DAP		1.77	45	442	0.29	1.89	51	443	0.32
B. pumilus + *M. ciceri*		1.82	61	498	0.34	1.95	57	498	0.41
Urea+ DAP		1.81	51	451	0.31	1.90	45	436	0.33
LSD		0.26	3.3	23.9	0.12	0.22	4.2	27.5	0.11
F value	Inoculation	128.5*	87.2*	78.5*	89.5*	71.5*	654.2*	90.4*	56.8*
	Fertilizer	6.2*	5.6*	3.7*	2.1*	3.5*	43.8*	3.5*	3.3*
	Interaction	2.1*	1.3*	1.5*	1.4*	2.1*	7.3*	1.4*	1.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of chickpea growth

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 45- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on nutrient uptake, seed yield and seed quality of chickpea plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		15	26	0.16	0.24	2.8	242	18	31	0.18	0.27	2.6	244
Urea (30kg/ha)		25	31	0.21	0.29	3.8	254	27	36	0.23	0.32	3.8	256
DAP (80 kg/ha)		24	29	0.24	0.31	4.1	257	29	33	0.29	0.38	4.2	258
B. pumilus		27	32	0.28	0.34	4.6	255	30	37	0.35	0.42	4.8	259
M. ciceri		29	37	0.26	0.29	4.3	261	38	42	0.29	0.36	4.7	262
B. pumilus +Urea		39	46	0.31	0.39	5.0	262	39	46	0.34	0.43	5.6	265
M. ciceri +DAP		38	44	0.34	0.41	5.3	262	39	46	0.41	0.47	5.5	268
B. pumilus + *M. ciceri*		43	51	0.39	0.45	6.2	265	42	51	0.50	0.55	6.4	271
Urea+ DAP		41	45	0.37	0.39	5.1	264	42	46	0.46	0.51	5.5	266
LSD		2.5	3.1	0.15	0.13	1.1	11.4	2.4	3.0	0.16	0.14	1.1	11.3
F value	Inoculation	143.5*	76.1*	85.5*	78.5*	72.5*	142.2*	89.4*	541.9*	91.5*	120.5*	106.5*	71.2*
	Fertilizer	17.5*	2.6*	4.4*	3.1*	2.5*	6.8*	3.2*	32.6*	5.8*	3.1*	4.2*	3.2*
	Interaction	5.1*	2.1*	1.2*	2.0*	1.1*	2.1*	2.6*	6.5*	1.2*	2.1*	2.1*	1.1*

Nutrient uptake, seed yield and seed protein were measured at 135 DAS

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 46-Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *M. ciceri* strain RG5 on growth of chickpea plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control		21.1	23.2	24.1	26.2	24.2	26.2	27.4	28.2
Urea (30kg/ha)		24.3	26.5	29.2	31.4	28.4	31.5	32.3	34.4
DAP (80 kg/ha)		25.5	24.8	31.3	32.3	29.5	30.3	34.2	35.5
Azotobacter		26.5	28.1	31.7	34.3	30.8	31.8	31.7	34.4
M. ciceri		31.3	31.3	33.2	37.5	31.3	34.1	34.5	36.2
Azotobacter +Urea		29.3	28.8	33.5	34.6	31.2	33.1	34.5	36.3
M. ciceri +DAP		31.2	32.3	36.3	38.3	33.3	35.6	37.6	39.2
Azotobacter + *M. ciceri*		31.2	32.4	37.3	39.5	34.2	36.5	38.2	39.2
Urea+ DAP		32.2	34.2	36.1	39.1	33.5	34.8	36.8	39.6
LSD		2.5	2.7	3.0	3.2	3.1	3.0	3.4	3.9
F value	Inoculation	67.5*	654.1*	76.5*	89.5*	77.5*	67.2*	90.4*	59.9*
	Fertilizer	4.2*	6.6*	3.1*	2.7*	4.5*	2.8*	4.1*	3.1*
	Interaction	1.3*	5.3*	1.6*	1.3*	2.5*	1.1*	1.2*	2.1*

[a]Control receiving neither fertilizers nor inoculant

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 47- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *M. ciceri* strain RG5on dry biomass of plant organs for chickpea plant

Treatments		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
[a]Control		0.93	0.98	2.11	2.92	1.12	1.18	2.20	3.00
Urea (30kg/ha)		1.23	1.31	2.35	3.45	1.34	1.38	2.45	3.62
DAP (80 kg/ha)		1.25	1.29	2.41	3.51	1.38	1.41	2.51	3.67
Azotobacter		1.29	1.35	2.44	3.53	1.39	1.41	2.52	3.75
M. ciceri		1.29	1.39	2.48	3.51	1.42	1.45	2.54	3.83
Azotobacter +Urea		1.36	1.46	2.52	3.62	1.41	1.44	2.80	3.91
M. ciceri +DAP		1.37	1.46	2.59	3.71	1.43	1.47	2.86	3.98
Azotobacter + *M. ciceri*		1.40	1.48	2.61	3.91	1.45	1.50	3.20	4.08
Urea+ DAP		1.41	1.47	2.61	3.81	1.45	1.47	3.12	4.01
LSD		0.21	0.25	0.30	0.39	0.14	0.15	0.31	0.39
F value	Inoculation	453.5*	86.1*	89.5*	87.5*	79.5*	87.2*	95.4*	56.9*
	Fertilizer	47.2*	3.6*	4.2*	3.2*	4.1*	3.8*	4.1*	4.2*
	Interaction	8.3*	1.3*	1.2*	1.2*	2.6*	2.3*	1.4*	2.1*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 48- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *M. ciceri* strain RG5 on photosynthetic and symbiotic attributes of chickpea plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		1.54	23	190	0.15	1.45	27	195	0.17
Urea (30kg/ha)		1.73	31	312	0.17	1.76	31	342	0.23
DAP (80 kg/ha)		1.71	33	323	0.18	1.78	32	345	0.25
Azotobacter		1.71	43	403	0.22	1.74	39	406	0.30
M. ciceri		1.69	52	450	0.28	1.82	54	464	0.36
Azotobacter +Urea		1.76	44	437	0.23	1.83	44	412	0.30
M. ciceri +DAP		1.77	45	442	0.29	1.89	51	443	0.32
Azotobacter + *M. ciceri*		1.79	57	479	0.33	1.91	53	478	0.35
Urea+ DAP		1.81	51	451	0.31	1.90	45	436	0.33
LSD		0.22	3.0	23.8	0.12	0.22	4.1	25.7	0.10
F value	Inoculation	65.5*	145.1*	87.5*	98.5*	87.5*	66.2*	134.4*	87.9*
	Fertilizer	4.2*	6.6*	4.2*	3.2*	3.4*	2.3*	4.5*	4.2*
	Interaction	2.0*	2.3*	1.6*	1.3*	2.1*	1.2*	1.7*	2.1*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of chickpea growth

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 49- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *M. ciceri* strain RG5 on nutrient uptake, seed yield and seed quality of chickpea plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		15	26	0.16	0.24	2.8	242	18	31	0.18	0.27	2.6	244
Urea (30kg/ha)		25	31	0.21	0.29	3.8	254	27	36	0.23	0.32	3.8	256
DAP (80 kg/ha)		24	29	0.24	0.31	4.1	257	29	33	0.29	0.38	4.2	258
Azotobacter		26	32	0.25	0.31	4.2	255	31	37	0.32	0.40	4.4	259
M. ciceri		29	37	0.26	0.29	4.3	261	38	42	0.29	0.36	4.7	262
Azotobacter +Urea		33	41	0.29	0.36	4.9	264	40	46	0.30	0.39	5.2	267
M. ciceri +DAP		38	44	0.34	0.41	5.3	262	39	46	0.41	0.47	5.5	268
Azotobacter + *M. ciceri*		40	46	0.37	0.41	5.5	266	43	50	0.46	0.50	6.0	269
Urea+ DAP		41	45	0.37	0.39	5.1	264	42	46	0.46	0.51	5.5	266
LSD		2.3	3.0	0.11	0.12	1.0	11.5	2.5	2.9	0.12	0.13	1.1	11.2
F value	Inoculation	135.5*	86.1*	96.5*	71.5*	322.5*	76.2*	87.4*	93.9*	90.5*	848.5*	236.5*	65.2*
	Fertilizer	5.5*	3.7*	4.4*	3.1*	6.5*	3.8*	5.5*	3.6*	2.8*	3.8*	32.5*	2.5*
	Interaction	2.3*	1.3*	1.8*	2.0*	4.1*	1.3*	2.1*	1.2*	1.3*	2.1*	6.1*	1.4*

Nutrient uptake, seed yield and seed protein were measured at 135 DAS

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

M. ciceri strain RG5 produced 232 µmol α- ketobutyrate/mg protein/h; Solubilized 75 µg/ml P; produced 64 µg/ml IAA at 100 µg/ml tryptophan; synthesized 13 and 28 µg/ml DHBA and SA, respectively; produced 26 µg/ml EPS; positive for ammonia and HCN;

Table 50-Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on growth of pea plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		15.7	20.3	21.3	22.7	23.0	27.3	27.7	30.3
Urea (20kg/ha)		19.3	23.6	23.7	26.7	25.7	29.0	31.7	33.3
DAP (90 kg/ha)		22.1	24.7	25.3	26.7	25.0	29.7	29.7	31.3
P. putida		22.6	25.7	26.7	28.7	26.0	28.3	28.7	32.6
R. leguminosarum		23.6	26.7	28.0	32.7	26.7	29.0	31.7	32.4
P. putida +Urea		28.3	30.7	29.3	31.3	28.0	28.3	32.6	34.3
R. leguminosarum +DAP		28.3	31.3	31.0	32.0	25.7	30.7	31.3	34.0
P. putida+ *R. leguminosarum*		29.7	32	32.3	32.7	28.3	29.0	33.5	34.3
Urea+ DAP		26.3	29.3	31.3	32.7	26.7	28.0	32.3	34.5
LSD		3.06	3.02	2.92	2.99	8.1	7.3	5.2	6.3
F value	Inoculation	118.5*	98.4*	176.5*	92.8*	118.3*	132.2*	115.4*	118.9*
	Fertilizer	4.5*	3.6*	5.2*	4.2*	8.5*	3.8*	3.5*	4.7*
	Interaction	2.1*	2.3*	2.4*	1.5*	2.6*	2.3*	1.8*	2.9*

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 51- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on dry biomass of plant organs for pea plant

Treatments		Dry biomass (g/plant)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.50	0.67	1.85	3.20	0.34	0.44	1.15	1.80
Urea (20kg/ha)		0.57	0.80	2.15	3.82	0.48	0.57	1.78	2.87
DAP (90 kg/ha)		0.58	0.81	2.38	3.84	0.59	0.60	1.83	2.23
P. putrid		0.67	0.82	2.34	3.76	0.52	0.68	1.87	2.60
R. leguminosarum		0.67	0.92	2.17	3.94	0.64	0.81	1.87	2.90
P. putida +Urea		0.75	0.92	2.86	4.06	0.59	0.84	2.09	3.21
R. leguminosarum +DAP		0.70	0.97	2.86	3.93	0.73	0.86	2.08	3.44
P. putida+ *R. leguminosarum* Urea+ DAP		0.78	1.05	2.81	4.01	0.77	1.05	2.16	3.11
LSD		0.73	0.96	2.87	4.21	0.84	0.98	2.30	3.57
F value	Inoculation	0.07	0.90	0.28	0.20	0.12	0.17	0.23	0.22
	Fertilizer	112.5*	188.1*	96.5*	132.5*	89.5*	142.2*	115.4*	128.9*
	Interaction	4.7*	3.8*	3.4*	4.2*	3.1*	3.5*	4.3*	3.7*
		2.1*	1.2*	2.4*	1.6*	1.1*	1.1*	1.8*	2.5*

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 52- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on photosynthetic and symbiotic attributes of pea plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.92	42	340	0.14	0.83	28	352	0.16
Urea (20kg/ha)		1.12	56	465	0.21	1.00	44	410	0.21
DAP (90 kg/ha)		1.16	56	460	0.22	1.11	48	416	0.22
P. putida		1.13	58	513	0.21	1.14	58	415	0.26
R. leguminosarum		1.24	64	596	0.23	1.19	65	414	0.22
P. putida +Urea		1.23	65	624	0.24	1.40	67	460	0.24
R. leguminosarum +DAP		1.37	73	628	0.22	1.70	70	516	0.25
P. putida+ *R. leguminosarum*		1.39	78	651	0.23	1.90	73	674	0.29
Urea+ DAP		1.36	76	648	0.24	1.80	61	608	0.26
LSD		0.20	13.9	39.4	0.05	0.22	15.6	44.7	0.06
F value	Inoculation	82.2*	118.1*	72.1*	87.5*	158.9*	99.1*	87.4*	111.3*
	Fertilizer	3.6*	4.1*	4.5*	3.2*	9.2*	3.9*	4.7*	4.2*
	Interaction	1.3*	2.1*	2.4*	1.7*	2.4*	1.7*	1.4*	1.3*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of chickpea growth

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 53- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on nutrient uptake, seed yield and seed quality of pea plant

Treatment		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		28	35	0.19	0.27	4.2	275	30	37	0.21	0.23	5.4	272
Urea (20kg/ha)		31	39	0.23	0.31	6.0	277	33	43	0.22	0.32	7.2	275
DAP (90 kg/ha)		33	40	0.25	0.33	6.3	280	34	41	0.22	0.31	7.5	285
P. putida		35	41	0.26	0.34	6.7	254	35	46	0.23	0.29	7.8	284
R. leguminosarum		35	41	0.27	0.35	6.5	282	37	48	0.24	0.34	8.2	284
P. putida +Urea		37	46	0.32	0.37	8.0	277	38	51	0.27	0.32	9.2	283
R. leguminosarum +DAP		35	43	0.36	0.37	7.5	277	39	51	0.29	0.34	8.7	283
P. putida+ *R. leguminosarum*		37	52	0.34	0.41	9.4	279	41	59	0.32	0.35	10.2	292
Urea+ DAP		38	47	0.35	0.38	8.4	280	40	53	0.36	0.39	9.2	291
LSD		6.5	10.2	0.07	0.07	1.7	21.3	8.4	9.6	0.61	0.05	1.3	12.9
F value	Inoculation	53.1*	123.3*	73.5*	129.3*	161.5*	52.8*	89.2*	85.9*	132.3*	98.3*	76.5*	59.1*
	Fertilizer	2.8*	3.9*	4.1*	5.2*	4.2*	5.1*	4.2*	4.1*	4.5*	3.6*	3.4*	4.5*
	Interaction	2.2*	2.1*	1.6*	3.1*	1.7*	2.7*	1.8*	1.8*	2.5*	1.6*	2.4*	2.3*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 54-Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on growth of pea plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		15.7	20.3	21.3	22.7	23.0	27.3	27.7	30.3
Urea (30kg/ha)		19.3	23.6	23.7	26.7	25.7	29.0	31.7	33.3
DAP (90 kg/ha)		22.1	24.7	25.3	26.7	25.0	29.7	29.7	31.3
B. pumilus		22.4	25.8	26.8	28.9	26.4	28.5	28.8	32.8
R. leguminosarum		23.6	26.7	28.0	32.7	26.7	29.0	31.7	32.4
B. pumilus +Urea		28.6	30.9	29.5	31.4	28.3	28.6	32.7	34.5
R. leguminosarum +DAP		28.3	31.3	31.0	32.0	25.7	30.7	31.3	34.0
B. pumilus + *R. leguminosarum*		29.9	32.8	32.5	32.8	28.5	29.4	33.7	34.4
Urea+ DAP		26.3	29.3	31.3	32.7	26.7	28.0	32.3	34.5
LSD		3.09	3.07	2.94	2.96	8.3	7.4	5.3	6.4
F value	Inoculation	128.5*	75.1*	654.5*	89.5*	76.5*	76.2*	85.4*	57.9*
	Fertilizer	6.2*	4.3*	54.2*	3.2*	3.5*	3.8*	3.5*	3.4*
	Interaction	2.6*	1.2*	6.4*	1.6*	2.4*	1.4*	1.4*	2.4*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 55- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on dry biomass of plant organs for pea plant

Treatment		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.50	0.67	1.85	3.20	0.34	0.44	1.15	1.80
Urea (30kg/ha)		0.57	0.80	2.15	3.82	0.48	0.57	1.78	2.87
DAP (90 kg/ha)		0.58	0.81	2.38	3.84	0.59	0.60	1.83	2.23
B. pumilus		0.69	0.85	2.36	3.78	0.54	0.71	1.89	2.62
R. leguminosarum		0.67	0.92	2.17	3.94	0.64	0.81	1.87	2.90
B. pumilus +Urea		0.78	0.95	2.88	4.09	0.61	0.86	2.11	3.23
R. leguminosarum +DAP		0.70	0.97	2.86	3.93	0.73	0.86	2.08	3.44
B. pumilus + *R. leguminosarum*		0.81	1.09	2.83	4.04	0.79	1.07	2.14	3.14
Urea+ DAP		0.73	0.96	2.87	4.21	0.84	0.98	2.30	3.57
LSD		0.07	0.12	0.26	0.21	0.11	0.18	0.24	0.24
F value	Inoculation	68.5*	98.1*	78.5*	802.5*	77.5*	67.2*	98.4*	56.9*
	Fertilizer	2.2*	4.6*	3.1*	42.2*	4.5*	4.8*	2.5*	3.2*
	Interaction	2.1*	1.2*	1.3*	5.1*	3.1*	1.4*	1.3*	2.3*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 56- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on photosynthetic and symbiotic attributes of pea plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.92	42	340	0.14	0.83	28	352	0.16
Urea (30kg/ha)		1.12	56	465	0.21	1.00	44	410	0.21
DAP (90 kg/ha)		1.16	56	460	0.22	1.11	48	416	0.22
B. pumilus		1.15	60	515	0.23	1.17	61	421	0.28
R. leguminosarum		1.24	64	596	0.23	1.19	65	414	0.22
B. pumilus +Urea		1.26	66	627	0.25	1.43	69	471	0.27
R. leguminosarum +DAP		1.37	73	628	0.22	1.70	70	516	0.25
B. pumilus + *R. leguminosarum*		1.43	79	651	0.26	1.97	76	685	0.31
Urea+ DAP		1.36	76	648	0.24	1.80	61	608	0.26
LSD		0.21	13.9	39.5	0.04	0.07	15.8	45.7	0.24
F value	Inoculation	54.5*	68.1*	86.5*	92.5*	91.5*	92.2*	915.4*	546.9*
	Fertilizer	6.2*	3.6*	2.2*	4.2*	4.5*	1.8*	4.9*	7.2*
	Interaction	2.1*	1.3*	1.4*	1.5*	2.1*	1.3*	1.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of pea growth

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 57- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on nutrient uptake, seed yield and seed quality of pea plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		28	35	0.19	0.27	4.2	275	30	37	0.21	0.23	5.4	272
Urea (30kg/ha)		31	39	0.23	0.31	6.0	277	33	43	0.22	0.32	7.2	275
DAP (90 kg/ha)		33	40	0.25	0.33	6.3	280	34	41	0.22	0.31	7.5	285
B. pumilus		36	42	0.27	0.35	6.6	287	36	47	0.26	0.31	8.1	285
R. leguminosarum		35	41	0.27	0.35	6.5	282	37	48	0.24	0.34	8.2	284
B. pumilus +Urea		37	47	0.33	0.39	8.2	281	39	52	0.29	0.34	9.5	286
R. leguminosarum +DAP		35	43	0.36	0.37	7.5	277	39	51	0.29	0.34	8.7	283
B. pumilus + *R. leguminosarum*		39	53	0.36	0.43	9.5	278	42	60	0.37	0.36	10.5	294
Urea+ DAP		38	47	0.35	0.38	8.4	280	40	53	0.36	0.39	9.2	291
LSD		6.5	10.1	0.06	0.06	1.8	21.3	8.5	9.4	0.63	0.06	1.4	13.1
F value	Inoculation	113.5*	34.3*	87.5*	98.5*	187.5*	876.2*	65.4*	143.9*	92.5*	118.5*	106.5*	43.2*
	Fertilizer	4.5*	3.6*	2.4*	3.5*	3.2*	43.8*	3.5*	3.1*	2.8*	2.8*	3.5*	3.5*
	Interaction	2.1*	2.3*	1.8*	2.1*	1.1*	2.3*	2.2*	1.6*	1.3*	2.1*	1.1*	1.2*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 58-Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *R. leguminosarum* strain RP2 on growth of pea plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		15.7	20.3	21.3	22.7	23.0	27.3	27.7	30.3
Urea (30kg/ha)		19.3	23.6	23.7	26.7	25.7	29.0	31.7	33.3
DAP (90 kg/ha)		22.1	24.7	25.3	26.7	25.0	29.7	29.7	31.3
Azotobacter		21.6	25.2	25.1	26.4	25.3	28.3	28.2	31.5
R. leguminosarum		23.6	26.7	28.0	32.7	26.7	29.0	31.7	32.4
Azotobacter +Urea		26.6	28.3	27.4	30.1	26.7	28.6	31.4	33.7
R. leguminosarum +DAP		28.3	31.3	31.0	32.0	25.7	30.7	31.3	34.0
Azotobacter + *R. leguminosarum*		27.8	31.2	31.5	31.6	27.1	28.2	31.8	34.1
Urea+ DAP		26.3	29.3	31.3	32.7	26.7	28.0	32.3	34.5
LSD		3.16	3.02	2.82	2.84	2.87	7.1	5.2	6.2
F value	Inoculation	765.5*	68.1*	96.5*	92.5*	81.5*	82.2*	876.4*	508.9*
	Fertilizer	32.2*	2.3*	4.2*	4.2*	2.5*	2.8*	43.5*	43.2*
	Interaction	2.1*	1.3*	1.4*	1.3*	2.1*	1.2*	6.9*	2.9*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 59- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *R. leguminosarum* strain RP2 on dry biomass of plant organs for pea plant

Treatment		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.50	0.67	1.85	3.20	0.34	0.44	1.15	1.80
Urea (30kg/ha)		0.57	0.80	2.15	3.82	0.48	0.57	1.78	2.87
DAP (90 kg/ha)		0.58	0.81	2.38	3.84	0.59	0.60	1.83	2.23
Azotobacter		0.64	0.80	2.30	3.73	0.49	0.66	1.84	2.57
R. leguminosarum		0.67	0.92	2.17	3.94	0.64	0.81	1.87	2.90
Azotobacter +Urea		0.71	0.91	2.83	4.03	0.57	0.81	2.04	3.19
R. leguminosarum +DAP		0.70	0.97	2.86	3.93	0.73	0.86	2.08	3.44
Azotobacter + *R. leguminosarum*		0.75	1.03	2.78	3.98	0.75	1.02	2.11	3.08
Urea+ DAP		0.73	0.96	2.87	4.21	0.84	0.98	2.30	3.57
LSD		0.05	0.16	0.15	0.21	0.12	0.18	0.21	0.18
F value	Inoculation	56.5*	768.1*	96.5*	89.5*	710.5*	69.2*	96.4*	55.2*
	Fertilizer	4.2*	44.6*	4.2*	2.7*	31.5*	1.8*	3.5*	3.1*
	Interaction	2.1*	7.3*	1.5*	1.3*	8.1*	1.3*	1.4*	2.5*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 60- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *R. leguminosarum* strain RP2 on photosynthetic and symbiotic attributes of pea plant

Treatment		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.92	42	340	0.14	0.83	28	352	0.16
Urea (30kg/ha)		1.12	56	465	0.21	1.00	44	410	0.21
DAP (90 kg/ha)		1.16	56	460	0.22	1.11	48	416	0.22
Azotobacter		1.11	62	501	0.20	1.12	60	400	0.24
R. leguminosarum		1.24	64	596	0.23	1.19	65	414	0.22
Azotobacter +Urea		1.20	61	607	0.22	1.38	64	445	0.22
R. leguminosarum +DAP		1.37	73	628	0.22	1.70	70	516	0.25
Azotobacter + *R. leguminosarum*		1.35	74	631	0.22	1.87	70	651	0.24
Urea+ DAP		1.36	76	648	0.24	1.80	61	608	0.26
LSD		0.18	13.9	35.2	0.04	0.05	15.6	44.3	0.21
F value	Inoculation	67.5*	89.1*	75.3*	92.5*	79.5*	86.2*	97.4*	57.9*
	Fertilizer	4.2*	3.6*	6.2*	4.2*	3.2*	3.8*	3.5*	3.2*
	Interaction	2.1*	1.2*	1.8*	1.3*	2.0*	1.3*	1.4*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of pea growth

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 61- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *R. leguminosarum* strain RP2 on nutrient uptake, seed yield and seed quality of pea plant

Treatment		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		28	35	0.19	0.27	4.2	275	30	37	0.21	0.23	5.4	272
Urea (30kg/ha)		31	39	0.23	0.31	6.0	277	33	43	0.22	0.32	7.2	275
DAP (90 kg/ha)		33	40	0.25	0.33	6.3	280	34	41	0.22	0.31	7.5	285
Azotobacter		33	40	0.26	0.33	6.4	280	35	44	0.24	0.27	7.9	285
R. leguminosarum		35	41	0.27	0.35	6.5	282	37	48	0.24	0.34	8.2	284
Azotobacter +Urea		35	44	0.30	0.36	7.6	281	38	49	0.25	0.30	9.0	282
R. leguminosarum +DAP		35	43	0.36	0.37	7.5	277	39	51	0.29	0.34	8.7	283
Azotobacter + *R. leguminosarum*		37	49	0.36	0.39	9.1	278	41	56	0.31	0.38	9.8	290
Urea+ DAP		38	47	0.35	0.38	8.4	280	40	53	0.36	0.39	9.2	291
LSD		6.3	9.2	0.05	0.05	1.5	21.1	8.4	9.3	0.58	0.04	1.5	11.2
F value	Inoculation	67.5*	51.1*	67.5*	75.5*	142.5*	81.2*	83.4*	143.9*	87.5*	126.5*	176.5*	87.2*
	Fertilizer	4.5*	2.6*	3.4*	3.2*	2.5*	3.0*	3.1*	7.6*	2.6*	3.5*	4.8*	3.1*
	Interaction	2.1*	1.3*	1.5*	2.0*	1.1*	2.3*	2.6*	1.8*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

R. leguminosarum strain RP2 produced ACC deaminase-238 µmol α- ketobutyrate/mg protein/h, Solubilized 81 µg/ml P; produced 65 µg/ml IAA at 100 µg/ml tryptophan; synthesized 12 and 24 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28µg/ml EPS; positive for ammonia and HCN

Table 62-Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on growth of greengram plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		18.5	21.2	22.8	25.2	17.2	20.3	20.7	23.3
Urea (25kg/ha)		25.2	27.8	27.2	29.6	26.4	28.2	27.8	30.1
DAP (85 kg/ha)		25.9	28.2	28.7	32.2	26.7	28.5	31.2	32.4
P. putida		28.2	30.9	31.2	33.2	28.1	31.2	31.8	34.2
Bradyrhizobium		29.5	31.6	32.7	34.3	30.2	32.5	32.2	34.7
P. putida +Urea		31.1	32.5	33.4	36.3	31.7	33.4	36.3	37.3
Bradyrhizobium +DAP		31.8	33.4	34.9	36.2	31.4	33.3	37.2	38.9
P. putida+ *Bradyrhizobium*		33.4	35.5	37.5	39.2	34.4	35.6	38.3	40.1
Urea+ DAP		32.4	34.6	35.6	37.5	33.5	34.5	37.8	39.7
LSD		2.9	3.1	3.4	3.6	2.9	3.2	3.4	3.6
F value	Inoculation	89.2*	143.5*	72.5*	72.5*	123.7*	72.1*	75.1*	168.9*
	Fertilizer	4.7*	4.2*	4.1*	3.1*	4.8*	3.6*	4.1*	6.2*
	Interaction	1.8*	3.4*	2.3*	1.7*	3.2*	1.8*	1.1*	1.3*

P. putida strain PSE3 produced ACC deaminase-625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 63- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on dry biomass of plant organs for greengram plant

Treatments		Dry biomass (g/plant)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		0.81	0.92	0.96	1.11	0.75	0.87	0.91	1.05
Urea (25kg/ha)		0.95	1.12	1.21	1.41	0.96	1.13	1.22	1.41
DAP (85 kg/ha)		1.02	1.15	1.25	1.42	1.03	1.15	1.26	1.43
P. putida		1.12	1.23	1.31	1.48	1.14	1.24	1.34	1.48
Bradyrhizobium		1.15	1.25	1.28	1.46	1.15	1.27	1.28	1.49
P. putida +Urea		1.18	1.31	1.46	1.65	1.19	1.33	1.45	1.65
Bradyrhizobium +DAP		1.23	1.32	1.49	1.72	1.23	1.32	1.49	1.72
P. putida+ *Bradyrhizobium*		1.32	1.35	1.58	1.78	1.35	1.36	1.59	1.82
Urea+ DAP		1.24	1.31	1.54	1.74	1.24	1.32	1.54	1.76
LSD		0.13	0.16	0.18	0.23	0.13	0.17	0.19	0.23
F value	Inoculation	175.3*	65.3*	96.2*	132.1*	51.4*	127.2*	85.4*	98.5*
	Fertilizer	6.8*	3.2*	5.7*	3.6*	3.9*	3.6*	3.5*	3.5*
	Interaction	1.8*	1.5*	2.2*	3.2*	2.3*	1.5*	1.2*	2.4*

P. putida strain PSE3 produced ACC deaminase-625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 64- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on photosynthetic and symbiotic attributes of greengram plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.83	24	86	0.08	0.89	21	75	0.07
Urea (25kg/ha)		0.92	34	134	0.12	0.98	33	124	0.12
DAP (85 kg/ha)		0.89	35	138	0.12	0.99	35	138	0.12
P. putida		0.94	46	175	0.14	1.05	46	175	0.15
Bradyrhizobium		0.97	52	191	0.17	1.07	55	191	0.17
P. putida +Urea		1.07	47	194	0.16	1.17	47	198	0.16
Bradyrhizobium +DAP		1.09	56	201	0.18	1.19	56	211	0.18
P. putida+ *Bradyrhizobium*		1.12	64	232	0.21	1.22	67	245	0.23
Urea+ DAP		1.11	51	198	0.16	1.21	51	201	0.17
LSD		0.06	4.5	12.6	0.03	0.07	4.6	12.8	0.03
F value	Inoculation	87.3*	54.6*	126.1*	62.4*	211.5*	82.7*	75.8*	108.7*
	Fertilizer	3.7*	3.1*	4.1*	3.1*	4.1*	3.4*	4.1*	4.7*
	Interaction	2.1*	1.5*	2.3*	1.5*	2.1*	1.7*	2.6*	1.8*

[a]Chlorophyll content in fresh foliage was measured at 50 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 50 DAS of greengram growth

P. putida strain PSE3 produced ACC deaminase-625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 65- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on nutrient uptake, seed yield and seed quality of greengram plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		32	48	0.22	0.32	3.2	256	28	44	0.20	0.29	3.4	254
Urea (25kg/ha)		39	57	0.32	0.39	4.5	265	40	58	0.27	0.37	4.7	266
DAP (85 kg/ha)		41	59	0.27	0.45	4.6	264	39	56	0.33	0.46	4.9	265
P. putida		44	62	0.36	0.46	5.4	267	45	62	0.37	0.48	5.8	269
Bradyrhizobium		46	65	0.34	0.45	5.1	262	47	67	0.34	0.45	5.9	267
P. putida +Urea		51	66	0.41	0.51	5.9	271	52	67	0.42	0.52	6.8	273
Bradyrhizobium +DAP		50	67	0.42	0.49	6.1	268	53	68	0.42	0.53	6.9	267
P. putida+ *Bradyrhizobium*		55	71	0.46	0.53	6.8	273	57	72	0.47	0.55	7.3	274
Urea+ DAP		52	69	0.44	0.51	6.5	272	54	69	0.46	0.54	7.1	272
LSD		2.9	3.4	0.04	0.05	0.6	3.5	2.9	3.4	0.04	0.05	0.6	3.5
F value	Inoculation	121.3*	51.8*	143.2*	72.4*	112.5*	62.5*	654.3*	129.5*	76.2*	125.3*	116.3*	542.2*
	Fertilizer	4.6*	3.9*	3.7*	4.8*	8.5*	4.2*	124.5*	7.6*	3.3*	4.2*	3.7*	123.5*
	Interaction	2.4*	2.3*	1.6*	3.2*	3.2*	1.7*	18.1*	3.2*	1.4*	2.0*	1.6*	18.9*

Nutrient uptake, seed yield and seed protein were measured at 80 DAS

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 66-Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on growth of greengram plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		18.5	21.2	22.8	25.2	17.2	20.3	20.7	23.3
Urea (25 kg/ha)		25.2	27.8	27.2	29.6	26.4	28.2	27.8	30.1
DAP (85 kg/ha)		25.9	28.2	28.7	32.2	26.7	28.5	31.2	32.4
B. pumilus		29.1	31.7	33.2	36.4	29.3	33.3	32.1	36.2
Bradyrhizobium		29.5	31.6	32.7	34.3	30.2	32.5	32.2	34.7
B. pumilus +Urea		32.2	34.3	35.3	37.2	33.2	35.3	38.2	39.4
Bradyrhizobium +DAP		31.8	33.4	34.9	36.2	31.4	33.3	37.2	38.9
B. pumilus + *Bradyrhizobium*		35.1	37.6	39.2	42.1	36.2	37.2	41.2	42.2
Urea+ DAP		32.4	34.6	35.6	37.5	33.5	34.5	37.8	39.7
LSD		2.7	3.2	3.1	3.4	3.1	3.4	3.5	3.4
F value	Inoculation	768.5*	48.1*	79.5*	80.5*	98.5*	66.2*	97.4*	70.9*
	Fertilizer	43.2*	2.6*	2.2*	2.1*	3.2*	2.2*	4.1*	3.2*
	Interaction	6.3*	1.1*	1.3*	1.1*	2.1*	1.3*	1.2*	2.1*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 67- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on dry biomass of plant organs for greengram plant

Treatments		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		0.81	0.92	0.96	1.11	0.75	0.87	0.91	1.05
Urea (25 kg/ha)		0.95	1.12	1.21	1.41	0.96	1.13	1.22	1.41
DAP (85 kg/ha)		1.02	1.15	1.25	1.42	1.03	1.15	1.26	1.43
B. pumilus		1.16	1.26	1.33	1.51	1.18	1.25	1.36	1.51
Bradyrhizobium		1.15	1.25	1.28	1.46	1.15	1.27	1.28	1.49
B. pumilus +Urea		1.21	1.34	1.49	1.69	1.24	1.37	1.48	1.67
Bradyrhizobium +DAP		1.23	1.32	1.49	1.72	1.23	1.32	1.49	1.72
B. pumilus + *Bradyrhizobium*		1.37	1.38	1.61	1.84	1.39	1.39	1.63	1.85
Urea+ DAP		1.24	1.31	1.54	1.74	1.24	1.32	1.54	1.76
LSD		0.11	0.14	0.21	0.21	0.14	0.18	0.21	0.25
F value	Inoculation	118.5*	128.1*	56.5*	86.5*	81.5*	68.2*	86.4*	52.9*
	Fertilizer	4.2*	5.6*	2.2*	2.4*	3.3*	2.7*	4.3*	3.1*
	Interaction	2.3*	1.3*	1.3*	1.2*	2.0*	1.1*	1.6*	2.1*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 68- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on photosynthetic and symbiotic attributes of greengram plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.83	24	86	0.08	0.89	21	75	0.07
Urea (25 kg/ha)		0.92	34	134	0.12	0.98	33	124	0.12
DAP (85 kg/ha)		0.89	35	138	0.12	0.99	35	138	0.12
B. pumilus		0.96	48	183	0.16	1.07	48	185	0.17
Bradyrhizobium		0.97	52	191	0.17	1.07	55	191	0.17
B. pumilus +Urea		1.09	49	207	0.18	1.19	50	211	0.18
Bradyrhizobium +DAP		1.09	56	201	0.18	1.19	56	211	0.18
B. pumilus + *Bradyrhizobium*		1.14	67	249	0.24	1.24	69	261	0.24
Urea+ DAP		1.11	51	198	0.16	1.21	51	201	0.17
LSD		0.05	4.1	12.9	0.04	0.08	4.4	13.4	0.04
F value	Inoculation	72.5*	65.1*	706.5*	89.5*	77.5*	87.2*	65.4*	57.9*
	Fertilizer	2.2*	2.1*	23.2*	2.2*	3.5*	2.8*	3.5*	3.2*
	Interaction	2.1*	1.3*	3.4*	1.1*	2.1*	1.3*	1.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 50 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 50 DAS of chickpea growth

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 69- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on nutrient uptake, seed yield and seed quality of greengram plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		32	48	0.22	0.32	3.2	256	28	44	0.20	0.29	3.4	254
Urea (25 kg/ha)		39	57	0.32	0.39	4.5	265	40	58	0.27	0.37	4.7	266
DAP (85 kg/ha)		41	59	0.27	0.45	4.6	264	39	56	0.33	0.46	4.9	265
B. pumilus		46	64	0.38	0.49	5.6	266	46	64	0.39	0.51	6.1	266
Bradyrhizobium		46	65	0.34	0.45	5.1	262	47	67	0.34	0.45	5.9	267
B. pumilus +Urea		53	68	0.43	0.54	6.2	274	53	68	0.47	0.56	7.2	275
Bradyrhizobium +DAP		50	67	0.42	0.49	6.1	268	53	68	0.42	0.53	6.9	267
B. pumilus + *Bradyrhizobium* Urea+ DAP		57	74	0.49	0.57	7.3	275	58	74	0.49	0.58	7.5	276
		52	69	0.44	0.51	6.5	272	54	69	0.46	0.54	7.1	272
LSD		2.7	3.1	0.05	0.05	0.5	3.3	2.7	3.1	0.06	0.06	0.7	3.3
F value	Inoculation	143.5*	88.1*	76.5*	654.5*	112.5*	88.2*	85.4*	126.9*	98.5*	126.5*	186.5*	72.2*
	Fertilizer	7.5*	4.6*	3.4*	43.2*	6.5*	3.8*	3.5*	3.2*	2.8*	3.8*	7.5*	3.5*
	Interaction	2.1*	2.3*	1.4*	8.1*	1.1*	2.3*	2.6*	1.5*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 80 DAS

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 70-Single and coinoculation effects phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *Bradyrhizobium* strain RB6 on growth of greengram plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		18.5	21.2	22.8	25.2	17.2	20.3	20.7	23.3
Urea (25 kg/hac)		25.2	27.8	27.2	29.6	26.4	28.2	27.8	30.1
DAP (85 kg/hac)		25.9	28.2	28.7	32.2	26.7	28.5	31.2	32.4
Azotobacter		27.8	30.2	31.5	32.4	26.7	30.8	31.5	34.7
Bradyrhizobium		29.5	31.6	32.7	34.3	30.2	32.5	32.2	34.7
Azotobacter +Urea		31.5	32.8	33.8	35.4	30.2	32.8	36.8	38.9
Bradyrhizobium +DAP		31.8	33.4	34.9	36.2	31.4	33.3	37.2	38.9
Azotobacter + *Bradyrhizobium*		32.8	34.3	36.2	38.6	33.8	35.9	34.3	39.5
Urea+ DAP		32.4	34.6	35.6	37.5	33.5	34.5	37.8	39.7
LSD		2.7	2.6	3.1	3.2	2.6	2.9	3.4	3.5
F value	Inoculation	58.5*	81.1*	656.5*	72.5*	81.5*	92.2*	115.4*	76.9*
	Fertilizer	2.2*	5.6*	43.2*	3.2*	3.5*	6.8*	6.5*	2.2*
	Interaction	2.3*	1.3*	6.4*	1.1*	2.1*	1.3*	1.6*	2.1*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 71- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *Bradyrhizobium* strain RB6 on dry biomass of plant organs for greengram plant

Treatments		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
[a]Control		0.81	0.92	0.96	1.11	0.75	0.87	0.91	1.05
Urea (25 kg/hac)		0.95	1.12	1.21	1.41	0.96	1.13	1.22	1.41
DAP (85 kg/hac)		1.02	1.15	1.25	1.42	1.03	1.15	1.26	1.43
Azotobacter		1.08	1.20	1.29	1.45	1.11	1.20	1.30	1.42
Bradyrhizobium		1.15	1.25	1.28	1.46	1.15	1.27	1.28	1.49
Azotobacter +Urea		1.15	1.27	1.41	1.61	1.15	1.31	1.41	1.61
Bradyrhizobium +DAP		1.23	1.32	1.49	1.72	1.23	1.32	1.49	1.72
Azotobacter + *Bradyrhizobium*		1.28	1.31	1.54	1.74	1.30	1.32	1.56	1.78
Urea+ DAP		1.24	1.31	1.54	1.74	1.24	1.32	1.54	1.76
LSD		0.11	0.14	0.15	0.20	0.11	0.14	0.16	0.21
F value	Inoculation	148.5*	98.1*	123.5*	72.5*	91.5*	82.2*	85.4*	76.9*
	Fertilizer	2.2*	3.6*	3.2*	2.2*	4.5*	2.8*	3.5*	4.2*
	Interaction	1.3*	1.3*	1.4*	1.1*	2.1*	1.7*	1.6*	2.5*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 72- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *Bradyrhizobium* strain RB6 on photosynthetic and symbiotic attributes of greengram plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.83	24	86	0.08	0.89	21	75	0.07
Urea (25 kg/hac)		0.92	34	134	0.12	0.98	33	124	0.12
DAP (85 kg/hac)		0.89	35	138	0.12	0.99	35	138	0.12
Azotobacter		0.91	44	161	0.13	1.04	44	161	0.14
Bradyrhizobium		0.97	52	191	0.17	1.07	55	191	0.17
Azotobacter +Urea		1.04	42	181	0.15	1.13	44	181	0.14
Bradyrhizobium +DAP		1.09	56	201	0.18	1.19	56	211	0.18
Azotobacter + *Bradyrhizobium*		1.10	61	212	0.19	1.20	65	224	0.21
Urea+ DAP		1.11	51	198	0.16	1.21	51	201	0.17
LSD		0.05	4.1	12.4	0.04	0.06	4.2	11.2	0.03
F value	Inoculation	78.5*	81.1*	76.2*	82.5*	91.5*	72.2*	195.4*	38.9*
	Fertilizer	3.2*	2.2*	3.2*	2.2*	4.5*	1.8*	14.5*	3.2*
	Interaction	2.3*	1.3*	1.4*	1.1*	2.1*	1.3*	1.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 50 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 50 DAS of chickpea growth

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 73- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *Bradyrhizobium* strain RB6 on nutrient uptake, seed yield and seed quality of greengram plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		32	48	0.22	0.32	3.2	256	28	44	0.20	0.29	3.4	254
Urea (25 kg/hac)		39	57	0.32	0.39	4.5	265	40	58	0.27	0.37	4.7	266
DAP (85 kg/hac)		41	59	0.27	0.45	4.6	264	39	56	0.33	0.46	4.9	265
Azotobacter		41	59	0.34	0.42	5.0	266	43	60	0.35	0.44	5.6	265
Bradyrhizobium		46	65	0.34	0.45	5.1	262	47	67	0.34	0.45	5.9	267
Azotobacter +Urea		49	64	0.39	0.49	5.5	270	50	65	0.40	0.50	6.6	270
Bradyrhizobium +DAP		50	67	0.42	0.49	6.1	268	53	68	0.42	0.53	6.9	267
Azotobacter + *Bradyrhizobium*		51	69	0.42	0.51	6.6	271	54	70	0.44	0.52	7.0	270
Urea+ DAP		52	69	0.44	0.51	6.5	272	54	69	0.46	0.54	7.1	272
LSD		2.5	3.1	0.05	0.05	0.7	3.3	2.7	3.4	0.04	0.06	0.5	3.1
F value	Inoculation	613.5*	82.1*	43.5*	79.5*	112.5*	86.2*	84.4*	123.9*	98.5*	127.5*	87.5*	76.2*
	Fertilizer	12.5*	4.6*	2.4*	3.2*	5.5*	6.8*	2.5*	1.6*	2.3*	3.2*	4.1*	3.1*
	Interaction	2.4*	1.3*	1.3*	2.1*	1.1*	2.3*	2.6*	1.1*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 80 DAS

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Bradyrhizobium strain RB6 produced ACC deaminase-211 µmol α- ketobutyrate/mg protein/h; Solubilized 73 µg/ml P; produced 74 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 25 µg/ml DHBA and SA, respectively; produced 15 µg/ml EPS; positive for ammonia and HCN;

Table 74-Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on growth of lentil plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		14.8	16.7	18.5	20.4	17.2	19.3	21.7	24.2
Urea (30kg/ha)		17.8	20.3	22.8	25.1	22.7	25.4	27.2	28.6
DAP (90 kg/ha)		18.1	21.2	24.9	26.2	23.5	26.2	28.4	29.8
P. putrid		21.2	24.1	25.7	27.2	25.6	27.1	28.3	30.9
Rhizobium		22.2	26.2	26.3	27.7	25.8	27.4	29.2	32.1
P. putida +Urea		21.7	25.2	29.3	31.2	28.5	29.1	31.2	33.3
Rhizobium +DAP		22.8	26.5	29.6	31.2	28.7	30.2	32.2	34.5
P. putida+ *Rhizobium*		25.2	28.3	31.2	34.3	31.4	33.5	33.2	36.1
Urea+ DAP		22.7	27.2	30.5	32.4	29.7	31.8	31.8	34.4
LSD		2.3	2.4	2.5	2.7	2.4	2.6	2.8	3.1
F value	Inoculation	378.2*	188.2*	762.4*	186.7*	371.4*	143.2*	915.4*	548.9*
	Fertilizer	123.2*	2.1*	5.2*	7.2*	8.5*	6.8*	3.5*	73.2*
	Interaction	12.3*	11.3*	31.4*	5.1*	12.2*	11.3*	5.6*	2.4*

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 75- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on dry biomass of plant organs for lentil plant

Treatments		Dry biomass (g/plant)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.52	0.57	1.21	1.25	0.62	0.65	1.34	1.36
Urea (30kg/ha)		0.61	0.68	1.62	1.71	0.78	0.84	1.85	1.98
DAP (90 kg/ha)		0.63	0.72	1.65	1.72	0.81	0.91	1.96	2.12
P. putida		0.67	0.72	1.67	1.77	0.92	0.97	2.01	2.14
Rhizobium		0.68	0.73	1.71	1.82	0.95	1.06	2.04	2.22
P. putida +Urea		0.69	0.75	1.89	2.06	0.98	1.07	2.13	2.36
Rhizobium +DAP		0.70	0.76	1.95	2.15	1.04	1.12	2.16	2.45
P. putida+ *Rhizobium*		0.74s	0.80	2.12	2.26	1.12	1.24	2.26	2.51
Urea+ DAP		0.71	0.77	2.01	2.14	1.03	1.11	2.17	2.44
LSD		0.12	0.12	0.21	0.26	0.12	0.17	0.27	0.32
F value	Inoculation	78.5*	88.1*	76.5*	82.5*	71.5*	62.2*	95.4*	58.9*
	Fertilizer	3.2*	2.6*	3.2*	2.2*	3.5*	2.8*	4.5*	3.2*
	Interaction	2.3*	1.3*	1.4*	1.1*	2.1*	1.3*	1.6*	2.5*

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 76- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on photosynthetic and symbiotic attributes of lentil plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.78	18	190	0.15	0.75	22	180	0.13
Urea (30kg/ha)		0.94	21	235	0.17	0.92	25	245	0.17
DAP (90 kg/ha)		1.01	22	245	0.19	1.01	26	265	0.18
P. putida		1.03	25	268	0.23	1.04	32	288	0.25
Rhizobium		1.05	28	285	0.25	1.07	36	315	0.27
P. putida +Urea		1.12	26	256	0.26	1.17	37	336	0.29
Rhizobium +DAP		1.14	29	296	0.27	1.24	39	336	0.31
P. putida+ *Rhizobium*		1.21	31	312	0.29	1.28	47	412	0.35
Urea+ DAP		1.17	29	294	0.25	1.25	41	334	0.29
LSD		0.10	2.8	12.5	0.05	0.11	3.4	19.2	0.06
F value	Inoculation	778.5*	88.1*	766.5*	852.5*	715.5*	602.2*	95.4*	59.9*
	Fertilizer	83.2*	2.6*	37.2*	25.2*	31.5*	29.8*	4.5*	9.2*
	Interaction	12.3*	1.3*	10.4*	15.1*	2.7*	17.3*	5.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of lentil growth

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN
Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 77- Single and coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on nutrient uptake, seed yield and seed quality of lentil plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		19	42	0.23	0.31	2.6	256	21	39	0.25	0.33	2.5	242
Urea (30kg/ha)		25	55	0.28	0.38	3.9	275	28	56	0.31	0.42	3.8	248
DAP (90 kg/ha)		27	57	0.32	0.41	4.1	279	25	59	0.35	0.46	4.5	251
P. putida		29	61	0.36	0.45	4.2	275	29	61	0.38	0.49	4.9	255
Rhizobium		31	65	0.36	0.47	5.3	281	32	67	0.41	0.51	5.3	256
P. putida +Urea		32	69	0.40	0.49	5.5	277	33	71	0.42	0.53	5.9	257
Rhizobium +DAP		35	71	0.42	0.51	5.8	284	35	72	0.42	0.57	5.8	255
P. putida+ *Rhizobium*		38	73	0.45	0.53	6.4	285	39	74	0.48	0.57	6.8	258
Urea+ DAP		37	71	0.44	0.53	6.2	285	37	71	0.47	0.56	6.5	258
LSD		4.6	6.3	0.06	0.07	1.1	6.5	4.6	6.2	0.06	0.07	1.3	6.5
F value	Inoculation	87.3*	54.6*	126.1*	62.4*	211.5*	82.7*	75.8*	108.7*	92.5*	128.5*	116.5*	72.2*
	Fertilizer	123.2*	2.1*	5.2*	7.2*	8.5*	6.8*	3.5*	73.2*	2.8*	3.8*	4.5*	3.5*
	Interaction	2.1*	2.3*	1.8*	2.1*	1.1*	2.3*	2.6*	1.5*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

P. putida strain PSE3 produced ACC deaminase 625 µmol α- ketobutyrate/mg protein/h, Solubilized 319 µg/ml P; produced 29 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 37 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 25 µg/ml EPS ; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 78-Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on growth of lentil plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		14.8	16.7	18.5	20.4	17.2	19.3	21.7	24.2
Urea (30 kg/ha)		17.8	20.3	22.8	25.1	22.7	25.4	27.2	28.6
DAP (90 kg/ha)		18.1	21.2	24.9	26.2	23.5	26.2	28.4	29.8
B. pumilus		22.3	24.5	28.1	28.8	27.6	29.7	27.7	30.2
Rhizobium		22.2	26.2	26.3	27.7	25.8	27.4	29.2	32.1
B. pumilus +Urea		22.8	25.4	30.5	32.1	30.4	30.9	32.4	33.7
Rhizobium +DAP		22.8	26.5	29.6	31.2	28.7	30.2	32.2	34.5
B. pumilus + *Rhizobium*		26.1	29.1	32.3	36.1	32.6	34.6	34.2	35.6
Urea+ DAP		22.7	27.2	30.5	32.4	29.7	31.8	31.8	34.4
LSD		2.3	2.2	2.6	2.9	2.5	2.2	2.6	2.9
F value	Inoculation	88.5*	54.1*	57.5*	654.5*	77.5*	621.2*	87.4*	57.9*
	Fertilizer	4.2*	2.6*	4.2*	32.2*	65.5*	26.4*	3.5*	2.2*
	Interaction	1.3*	1.2*	1.6*	1.4*	3.1*	5.6*	1.4*	2.1*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 79- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on dry biomass of plant organs for lentil plant

Treatments		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.52	0.57	1.21	1.25	0.62	0.65	1.34	1.36
Urea (30 kg/ha)		0.61	0.68	1.62	1.71	0.78	0.84	1.85	1.98
DAP (90 kg/ha)		0.63	0.72	1.65	1.72	0.81	0.91	1.96	2.12
B. pumilus		0.69	0.72	1.71	1.78	0.94	0.96	2.04	2.16
Rhizobium		0.68	0.73	1.71	1.82	0.95	1.06	2.04	2.22
B. pumilus +Urea		0.74	0.76	1.88	2.08	0.99	1.09	2.15	2.39
Rhizobium +DAP		0.70	0.76	1.95	2.15	1.04	1.12	2.16	2.45
B. pumilus + *Rhizobium*		0.76	0.81	2.17	2.29	1.15	1.26	2.29	2.55
Urea+ DAP		0.71	0.77	2.01	2.14	1.03	1.11	2.17	2.44
LSD		0.13	0.11	0.22	0.25	0.11	0.19	0.25	0.31
F value	Inoculation	86.5*	95.1*	704.5*	92.5*	654.5*	79.2*	98.4*	74.9*
	Fertilizer	5.2*	4.6*	33.2*	5.2*	36.5*	2.3*	3.5*	3.1*
	Interaction	2.5*	1.6*	7.4*	3.1*	2.4*	1.2*	1.4*	2.5*

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN
Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 80- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on photosynthetic and symbiotic attributes of lentil plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.78	18	190	0.15	0.75	22	180	0.13
Urea (30 kg/ha)		0.94	21	235	0.17	0.92	25	245	0.17
DAP (90 kg/ha)		1.01	22	245	0.19	1.01	26	265	0.18
B. pumilus		1.05	27	278	0.24	1.05	35	307	0.26
Rhizobium		1.05	28	285	0.25	1.07	36	315	0.27
B. pumilus +Urea		1.14	28	272	0.28	1.19	39	351	0.29
Rhizobium +DAP		1.14	29	296	0.27	1.24	39	336	0.31
B. pumilus + *Rhizobium*		1.23	33	327	0.31	1.31	48	415	0.37
Urea+ DAP		1.17	29	294	0.25	1.25	41	334	0.29
LSD		0.11	2.5	14.1	0.06	0.12	3.3	19.1	0.07
F value	Inoculation	568.5*	68.1*	703.5*	84.5*	76.5*	85.2*	99.4*	518.9*
	Fertilizer	43.2*	3.6*	31.2*	3.2*	3.5*	2.9*	4.1*	44.2*
	Interaction	4.3*	2.3*	3.4*	3.1*	2.1*	1.4*	1.3*	6.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of lentil growth

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

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Table 81- Single and coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on nutrient uptake, seed yield and seed quality of lentil plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		19	42	0.23	0.31	2.6	256	21	39	0.25	0.33	2.5	242
Urea (30 kg/ha)		25	55	0.28	0.38	3.9	275	28	56	0.31	0.42	3.8	248
DAP (90 kg/ha)		27	57	0.32	0.41	4.1	279	25	59	0.35	0.46	4.5	251
B. pumilus		29	63	0.39	0.47	4.3	276	30	63	0.41	0.52	5.1	254
Rhizobium		31	65	0.36	0.47	5.3	281	32	67	0.41	0.51	5.3	256
B. pumilus +Urea		33	69	0.42	0.51	5.7	278	34	71	0.43	0.55	6.2	259
Rhizobium +DAP		35	71	0.42	0.51	5.8	284	35	72	0.42	0.57	5.8	255
B. pumilus + *Rhizobium*		39	74	0.47	0.55	6.7	289	39	76	0.51	0.58	6.9	258
Urea+ DAP		37	71	0.44	0.53	6.2	285	37	71	0.47	0.56	6.5	258
LSD		4.5	6.3	0.07	0.06	1.2	6.7	4.4	6.1	0.07	0.06	1.2	6.5
F value	Inoculation	63.5*	801.1*	85.5*	79.5*	112.5*	812.2*	87.4*	123.9*	92.5*	128.5*	166.5*	85.2*
	Fertilizer	2.5*	32.1*	3.4*	3.2*	3.5*	41.8*	3.2*	3.6*	2.8*	3.8*	8.5*	3.5*
	Interaction	2.1*	2.3*	1.8*	2.1*	1.1*	2.3*	2.4*	1.5*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

B. pumilus strain ES3 produced ACC deaminase-345 µmol α- ketobutyrate/mg protein/h, Solubilized 265 µg/ml P; produced 45 µg/ml IAA at 100 µg/ml tryptophan; synthesized 14 and 31 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 18µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 82-Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing and *Rhizobium* strain RV9 on growth of lentil plant

Treatments		Length/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		14.8	16.7	18.5	20.4	17.2	19.3	21.7	24.2
Urea (30 kg/hac)		17.8	20.3	22.8	25.1	22.7	25.4	27.2	28.6
DAP (90 kg/hac)		18.1	21.2	24.9	26.2	23.5	26.2	28.4	29.8
Azotobacter		20.6	22.8	24.2	26.1	23.9	27.5	27.4	30.4
Rhizobium		22.2	26.2	26.3	27.7	25.8	27.4	29.2	32.1
Azotobacter +Urea		21.7	24.8	28.7	30.5	27.9	29.4	30.9	32.6
Rhizobium +DAP		22.8	26.5	29.6	31.2	28.7	30.2	32.2	34.5
Azotobacter + *Rhizobium*		23.8	26.9	30.4	32.5	30.8	31.4	31.6	34.2
Urea+ DAP		22.7	27.2	30.5	32.4	29.7	31.8	31.8	34.4
LSD		2.1	2.2	2.3	2.4	2.2	2.4	2.4	2.8
F value	Inoculation	88.5*	954.1*	78.5*	82.5*	78.5*	67.2*	932.4*	58.4*
	Fertilizer	4.2*	42.6*	4.2*	2.2*	4.5*	2.9*	94.5*	3.5*
	interacttion	2.3*	5.3*	1.4*	1.1*	2.3*	1.4*	8.6*	2.1*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 83- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *Rhizobium* strain RV9 on dry biomass of plant organs for lentil plant

Treatments		Dry biomass/plant (cm)							
		Pot trials				Field trials			
		Root		Shoot		Root		Shoot	
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
[a]Control		0.52	0.57	1.21	1.25	0.62	0.65	1.34	1.36
Urea (30 kg/hac)		0.61	0.68	1.62	1.71	0.78	0.84	1.85	1.98
DAP (90 kg/hac)		0.63	0.72	1.65	1.72	0.81	0.91	1.96	2.12
Azotobacter		0.64	0.70	1.64	1.75	0.90	0.94	2.00	2.11
Rhizobium		0.68	0.73	1.71	1.82	0.95	1.06	2.04	2.22
Azotobacter +Urea		0.65	0.71	1.85	2.01	0.95	1.06	2.10	2.32
Rhizobium +DAP		0.70	0.76	1.95	2.15	1.04	1.12	2.16	2.45
Azotobacter + *Rhizobium*		0.71	0.78	2.04	2.20	1.10	1.20	2.23	2.49
Urea+ DAP		0.71	0.77	2.01	2.14	1.03	1.11	2.17	2.44
LSD		0.11	0.12	0.19	0.22	0.10	0.16	0.25	0.31
F value	Inoculation	118.5*	98.1*	76.5*	802.5*	712.5*	69.2*	99.4*	69.9*
	Fertilizer	13.2*	3.6*	3.2*	32.2*	53.5*	5.8*	4.5*	3.2*
	Interaction	3.3*	1.3*	1.4*	3.1*	5.1*	1.3*	1.6*	2.5*

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 84- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *Rhizobium* strain RV9 on photosynthetic and symbiotic attributes of lentil plant

Treatments		Pot trials				Field trials			
		[a]Chlorophyll content (mg/g)	Symbiotic attributes			[a]Chlorophyll content (mg/g)	Symbiotic attributes		
			[b]Nodules No./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$		[b]Nodules no./ plant	[b]Nodule biomass (mg/ plant)	[b]Leghaemoglobin mM (g f m)$^{-1}$
[a]Control		0.78	18	190	0.15	0.75	22	180	0.13
Urea (30 kg/hac)		0.94	21	235	0.17	0.92	25	245	0.17
DAP (90 kg/hac)		1.01	22	245	0.19	1.01	26	265	0.18
Azotobacter		1.00	23	245	0.20	1.02	30	272	0.23
Rhizobium		1.05	28	285	0.25	1.07	36	315	0.27
Azotobacter +Urea		1.08	24	241	0.23	1.15	35	321	0.24
Rhizobium +DAP		1.14	29	296	0.27	1.24	39	336	0.31
Azotobacter + *Rhizobium*		1.19	29	300	0.25	1.25	44	387	0.31
Urea+ DAP		1.17	29	294	0.25	1.25	41	334	0.29
LSD		0.11	2.5	12.5	0.04	0.10	3.1	17.5	0.05
F value	Inoculation	718.5*	98.1*	706.5*	82.5*	71.6*	622.2*	99.4*	59.9*
	Fertilizer	73.2*	3.6*	33.2*	2.7*	13.5*	2.8*	4.7*	4.2*
	interacttion	12.3*	1.3*	1.4*	1.5*	2.1*	1.8*	1.6*	2.5*

[a]Chlorophyll content in fresh foliage was measured at 60 days; [b]Nodule no., [b]Nodule dry biomass and [b]Leghaemoglobin content in fresh nodule was determined at 90 DAS of lentil growth

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

Table 85- Single and coinoculation effects of phosphate solubilizing *Azotobacter* strain AZ19 and ACC deaminase producing *Rhizobium* strain RV9 on nutrient uptake, seed yield and seed quality of lentil plant

Treatments		Pot trials						Field trials					
		Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)	Nutrient uptake				Seed yield (g/plant)	Seed proteins (mg/g)
		N content (mg/g)		P content (mg/g)				N content (mg/g)		P content (mg/g)			
		Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot		
[a]Control		19	42	0.23	0.31	2.6	256	21	39	0.25	0.33	2.5	242
Urea (30 kg/hac)		25	55	0.28	0.38	3.9	275	28	56	0.31	0.42	3.8	248
DAP (90 kg/hac)		27	57	0.32	0.41	4.1	279	25	59	0.35	0.46	4.5	251
Azotobacter		27	59	0.33	0.41	4.0	275	27	60	0.34	0.47	4.5	256
Rhizobium		31	65	0.36	0.47	5.3	281	32	67	0.41	0.51	5.3	256
Azotobacter +Urea		30	66	0.38	0.45	5.3	274	32	69	0.40	0.51	5.6	256
Rhizobium +DAP		35	71	0.42	0.51	5.8	284	35	72	0.42	0.57	5.8	255
Azotobacter + *Rhizobium*		36	70	0.42	0.50	6.2	278	37	72	0.45	0.55	6.5	256
Urea+ DAP		37	71	0.44	0.53	6.2	285	37	71	0.47	0.56	6.5	258
LSD		4.4	6.1	0.05	0.06	1.0	6.7	4.2	5.8	0.05	0.06	1.1	6.1
F value	Inoculation	163.5*	821.1*	86.5*	77.5*	112.5*	92.2*	875.4*	123.9*	92.5*	128.5*	116.5*	72.2*
	Fertilizer	2.5*	3.6*	3.7*	3.2*	3.3*	3.8*	83.5*	3.6*	2.8*	3.8*	4.5*	3.5*
	Interaction	2.1*	2.3*	1.8*	2.1*	1.1*	2.3*	2.6*	1.5*	1.3*	2.2*	2.1*	1.3*

Nutrient uptake, seed yield and seed protein were measured at 120 DAS

Azotobacter strain AZ19 solubilized 115 µg/ml P; produced 96 µg/ml IAA at 100 µg/ml tryptophan; synthesized 15 and 28 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 19 µg/ml EPS; positive for ammonia and HCN

Rhizobium strain RV9 produced ACC deaminase-185 µmol α- ketobutyrate/mg protein/h, Solubilized 123 µg/ml P; produced 92 µg/ml IAA at 100 µg/ml tryptophan; synthesized 16 and 29 µg/ml 2,3 dihydroxy benzoic acid (DHBA), and salicylic acid (SA), respectively; produced 28 µg/ml EPS; positive for ammonia and HCN

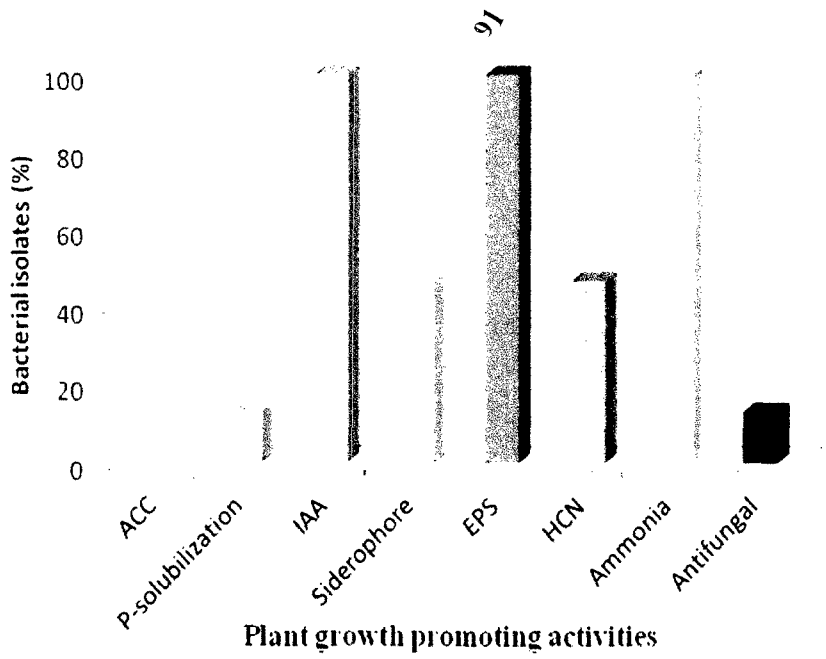

Fig. 8- Plant growth promoting activities of *Mesorhizobium* (N=15) isolated from chickpea nodules

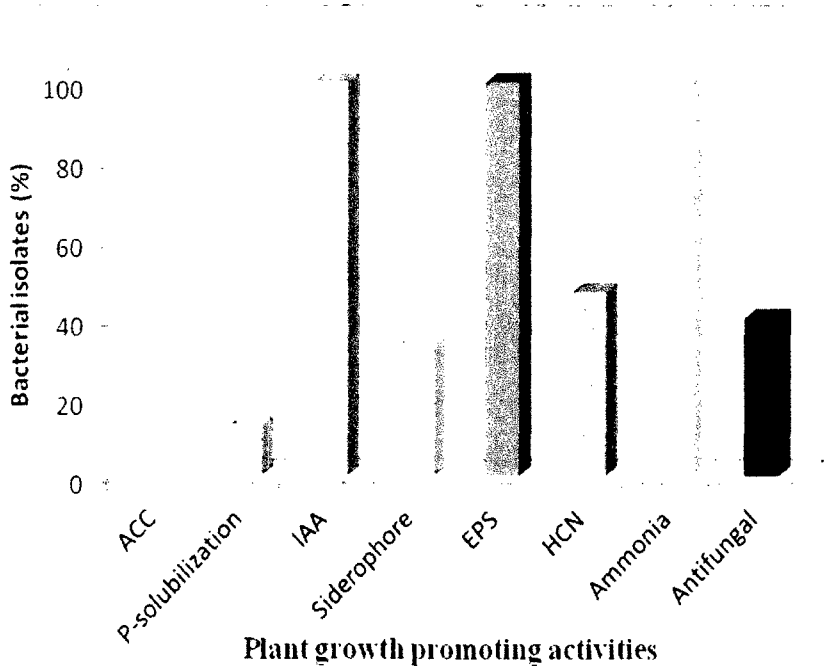

Fig. 9 - Plant growth promoting activities of *Rhizobium* (N=15) isolated from pea nodules

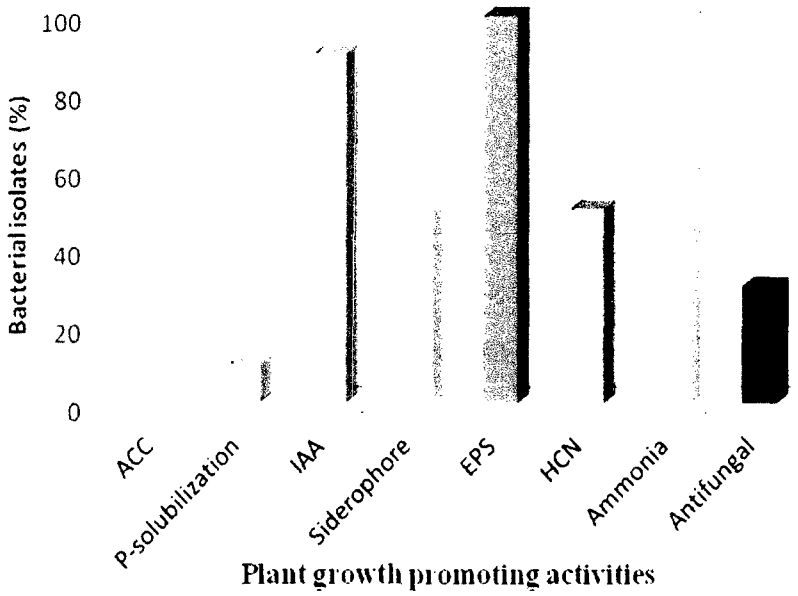

Fig. 10- Plant growth promoting activities of *Bradyrhizobium* (N=10) isolated from greengram nodules

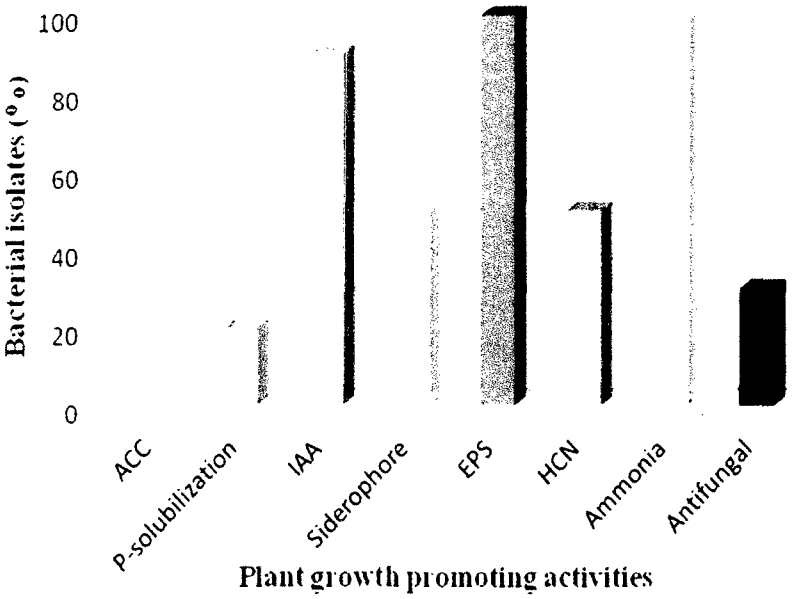

Fig. 11- Plant growth promoting activities of *Rhizobium* (N=10) isolated from lentil nodules

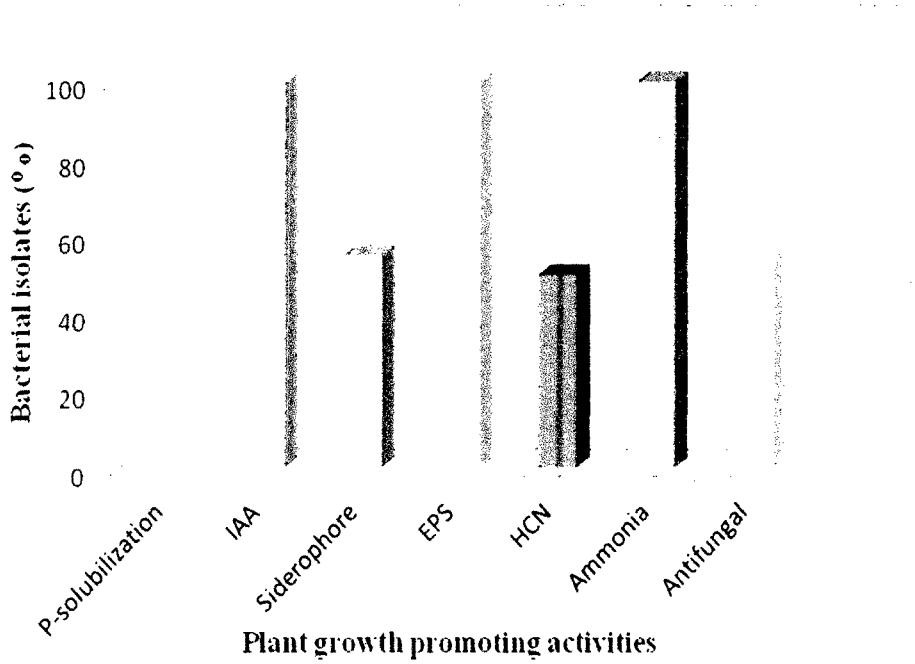

Fig 12 - Plant growth promoting activities of *Azotobacter* (N=20) isolated from rhizospheric soils

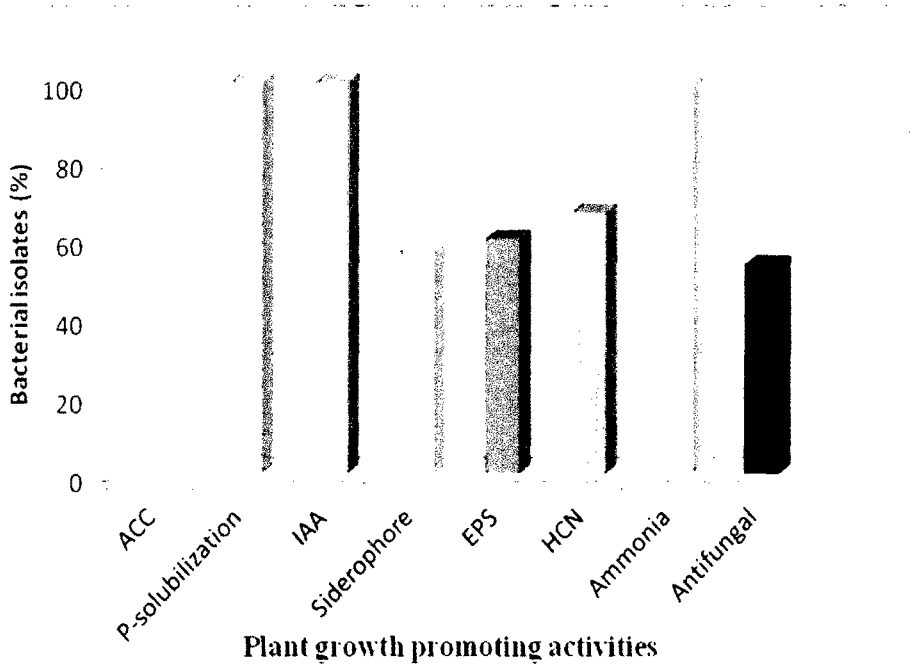

Fig. 13– Plant growth promoting activities of P-solubilizer (N=30) isolated from rhizospheric soils

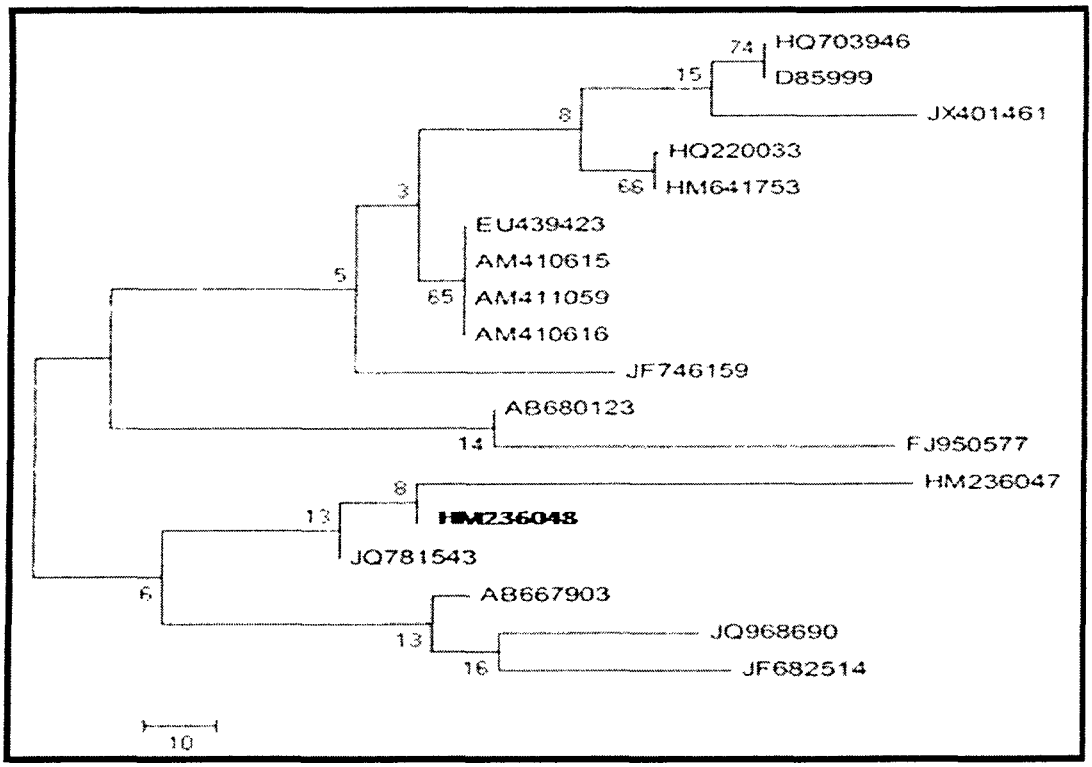

Fig. 14 -phylogenetic tree constructed from the 16S rRNA gene sequence of *P. putida* (GenBank accession no. HM236947 and HM236948) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

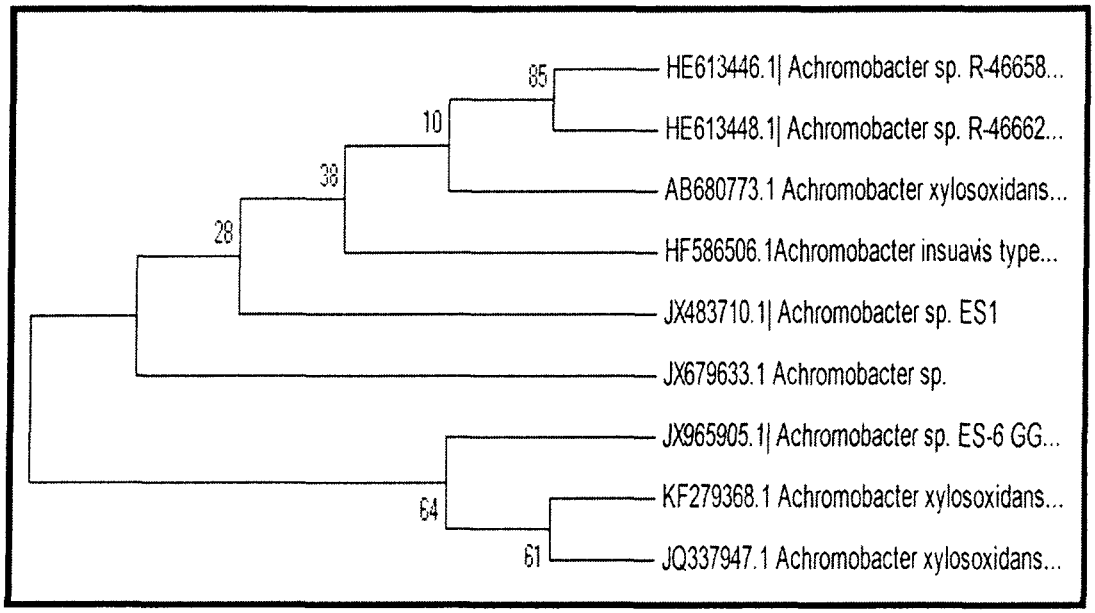

Fig. 15 -phylogenetic tree constructed from the 16S rRNA gene sequence of *Achromobacter* (GenBank accession no. JX483710, JX965905) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

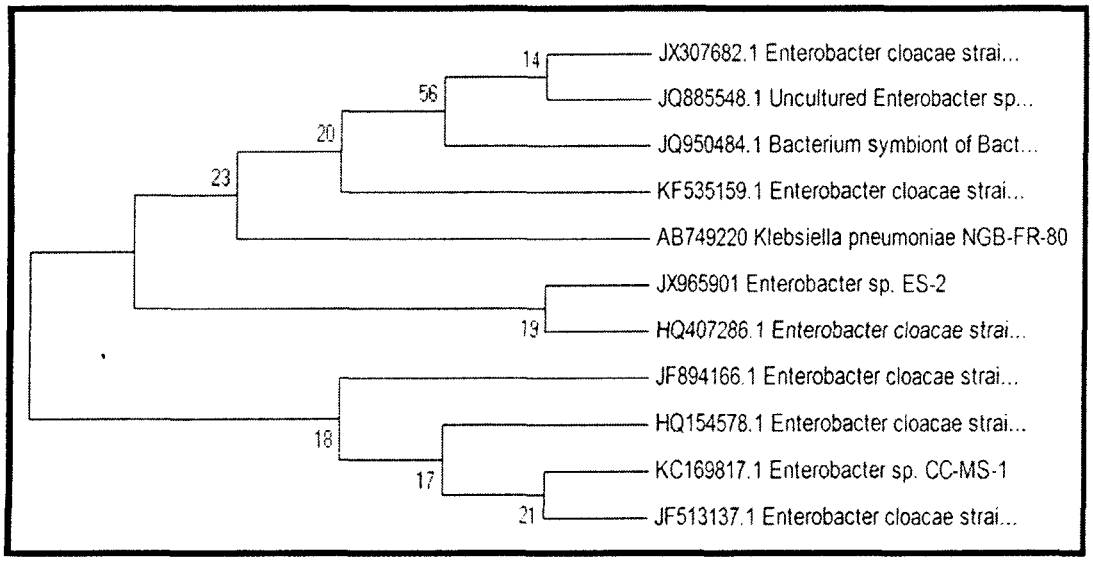

Fig. 16 phylogenetic tree constructed from the 16S rRNA gene sequence of *Enterobacter* (GenBank accession no. JX965901) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

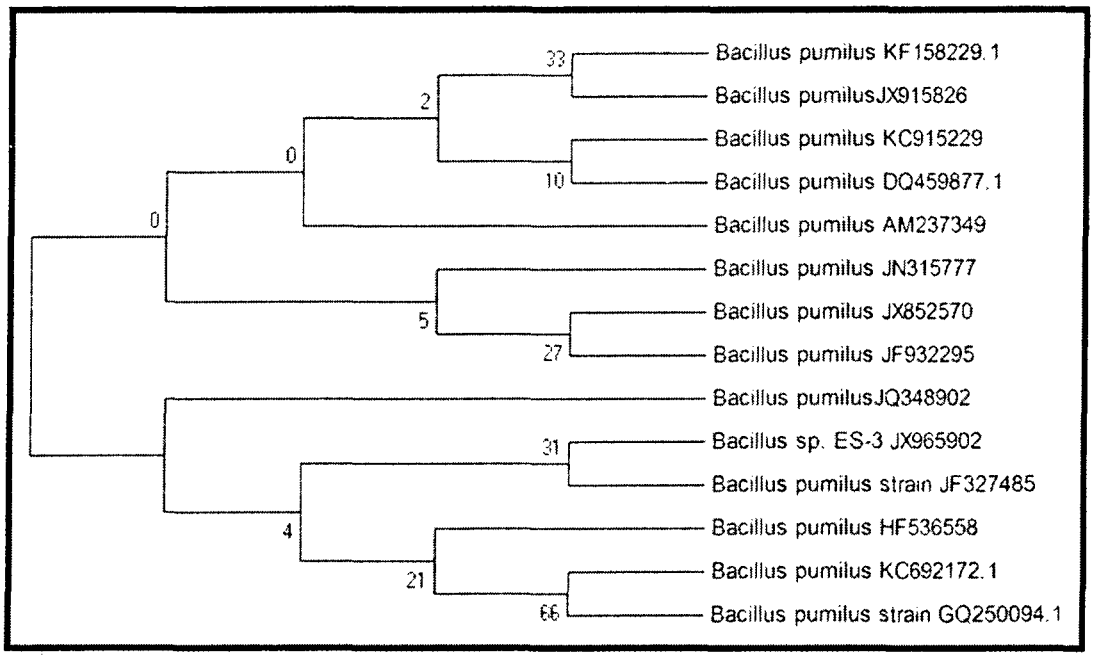

Fig. 17 phylogenetic tree constructed from the 16S rRNA gene sequence of *Bacillus pumilus* (GenBank accession no. JX965902) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

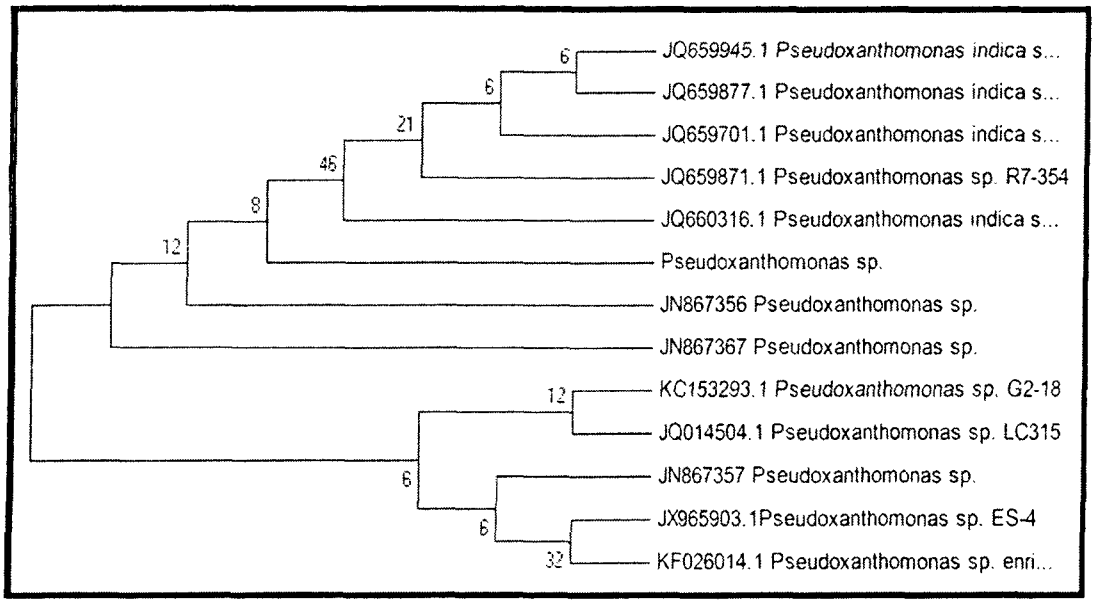

Fig. 18- phylogenetic tree constructed from the 16S rRNA gene sequence of *Pseudoxanthomonas* (GenBank accession no. JX965903) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

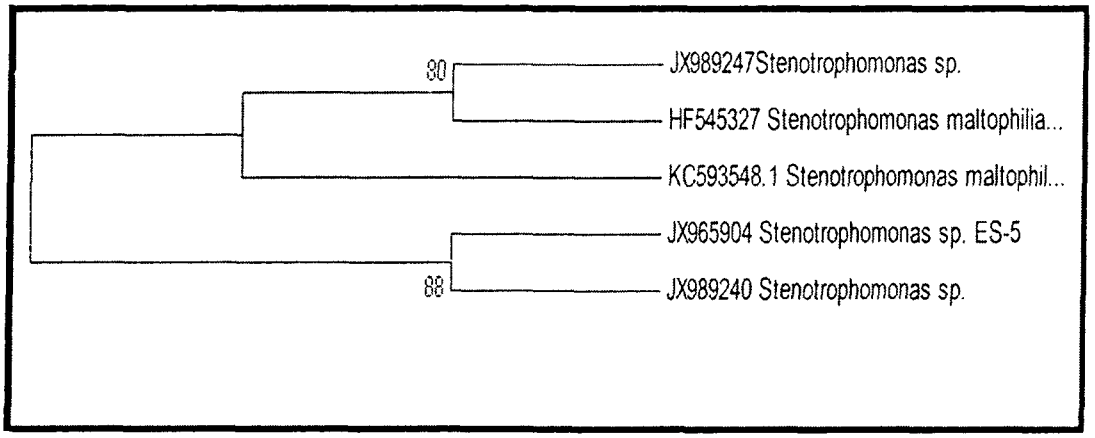

Fig. 19- phylogenetic tree constructed from the 16S rRNA gene sequence of *Stenotrophomonas* (GenBank accession no. JX965904) and related organism using NCBI BLASTn analysis and neighbour-joining algorithm from the alignment (Clustal W) of nucleotides sequence by MEGA4.1

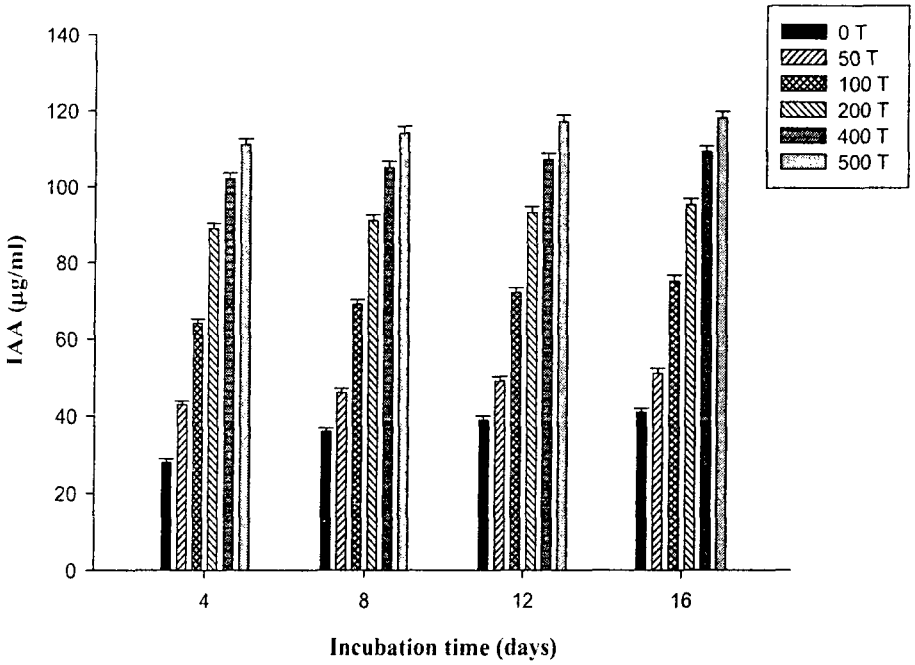

Fig. 20 Indole acetic acid production by *Mesorhizobium ciceri* strain grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

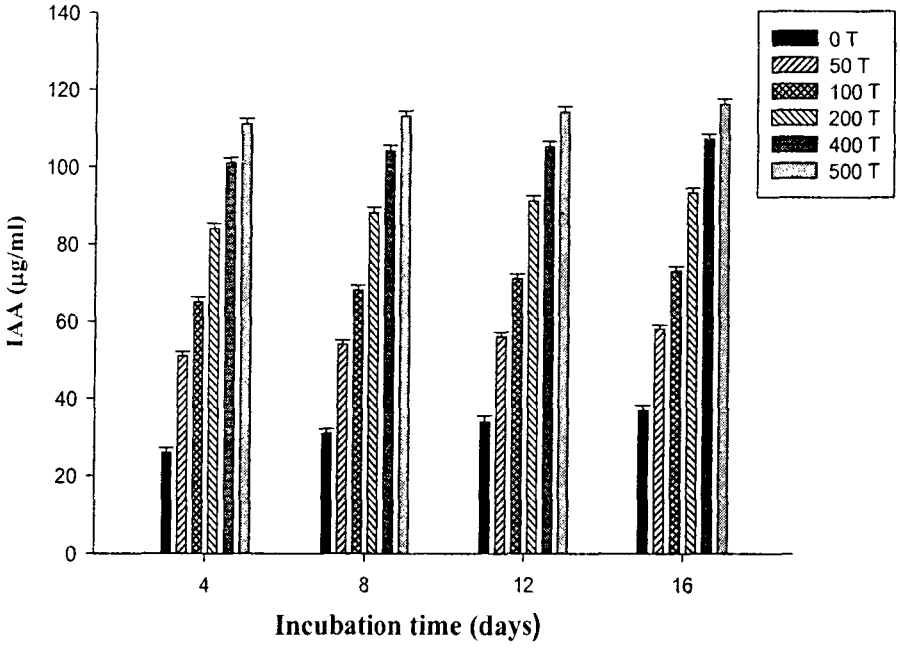

Fig. 21 Indole acetic acid production by RG5 *R. leguminosarum* strain RP2 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

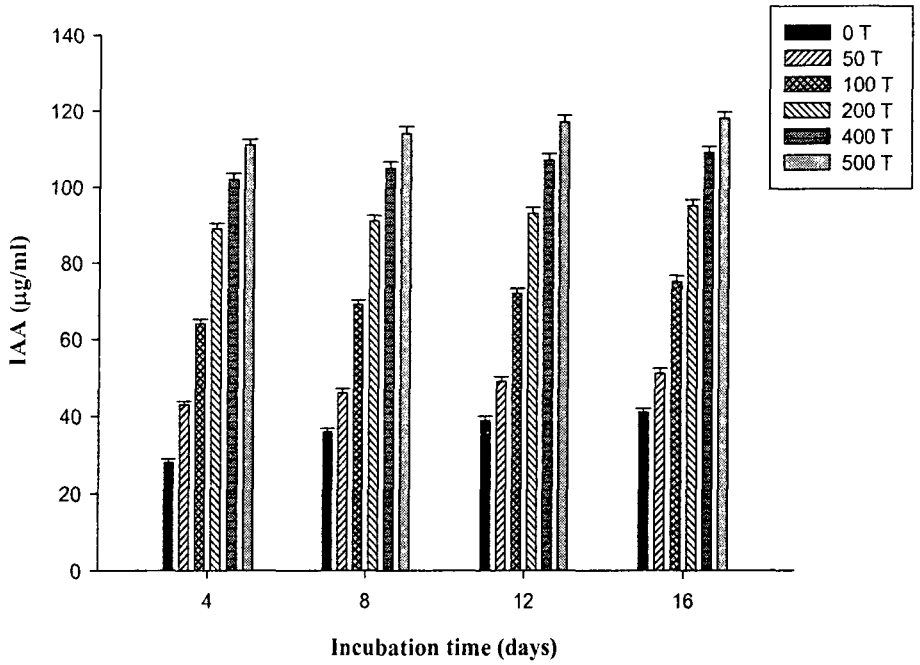

Fig 22 Indole acetic acid production by *Bradyrhizobium* strain RB6 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

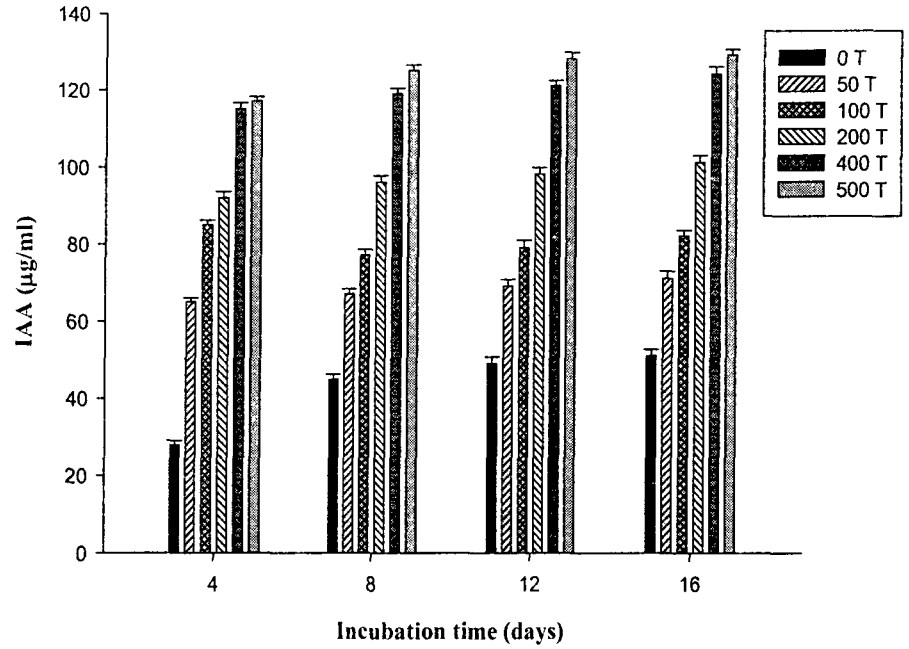

Fig. 23- Indole acetic acid production by *Rhizobium* strain RV9 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

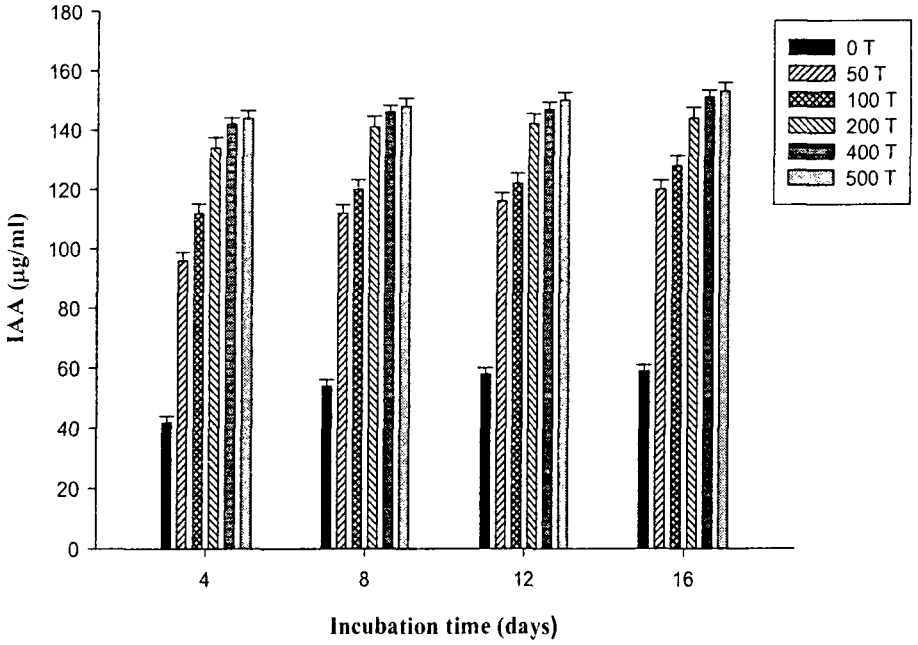

Fig. 24 Indole acetic acid production by *Azotobacter* strain AZ19 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

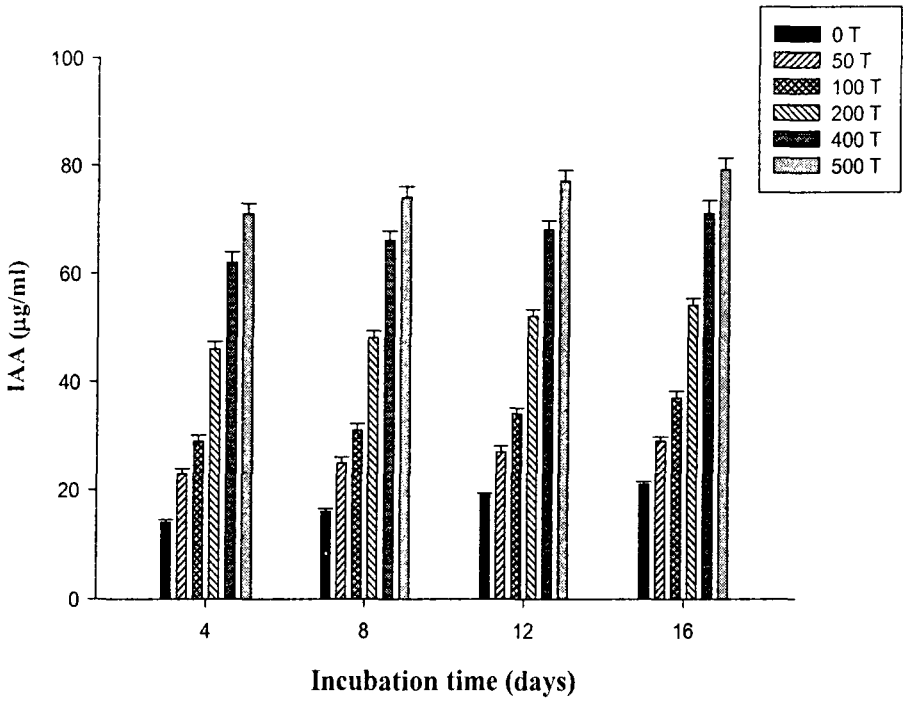

Fig. 25 Indole acetic acid production by *P. putida* strain PSE3 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

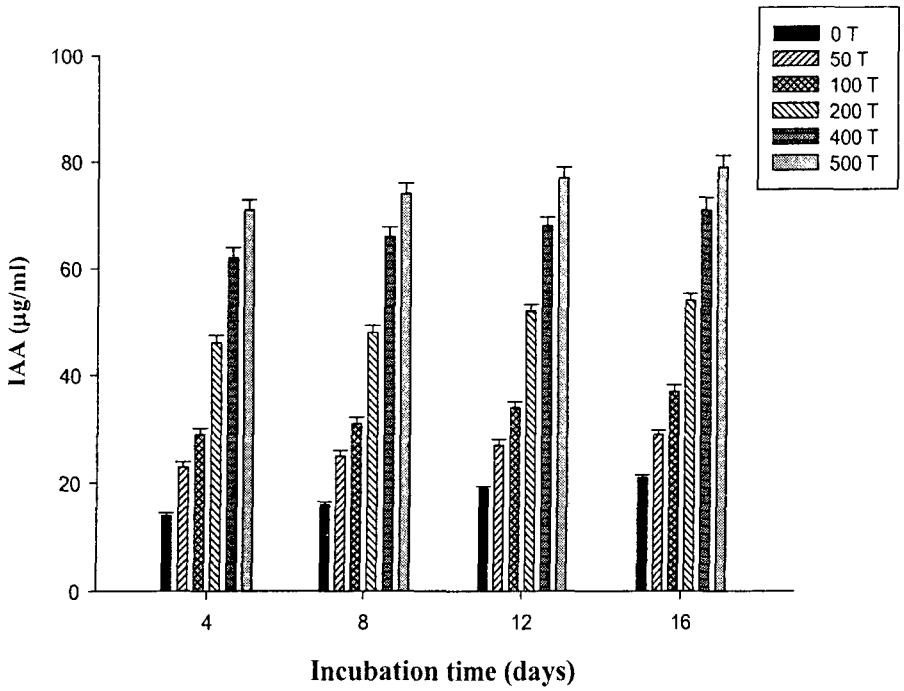

Fig. 26- Indole acetic acid production by *P. putida* strain PSE5 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

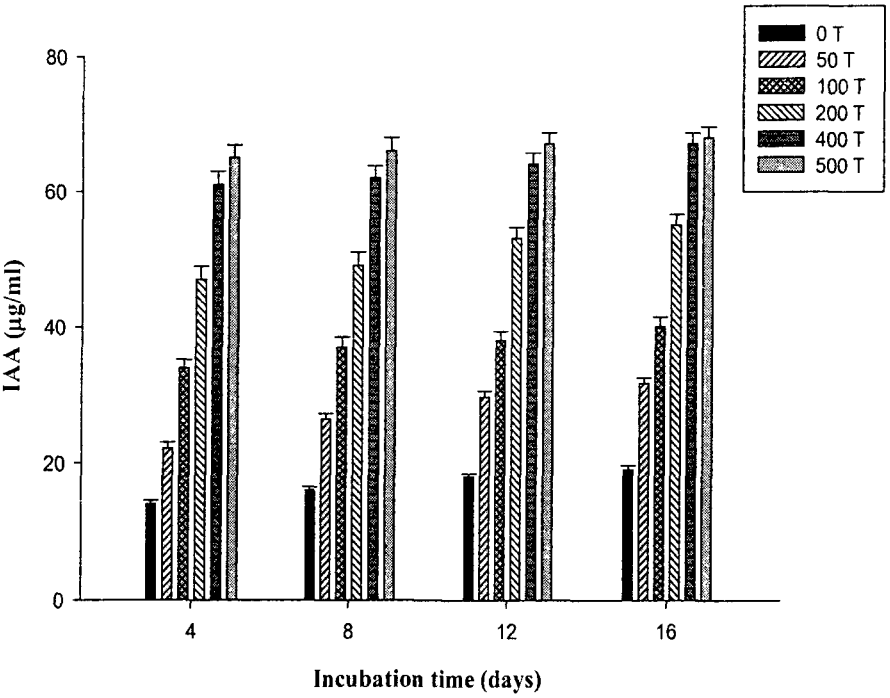

Fig. 27- Indole acetic acid production by *Achromobacter* strain ES1 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

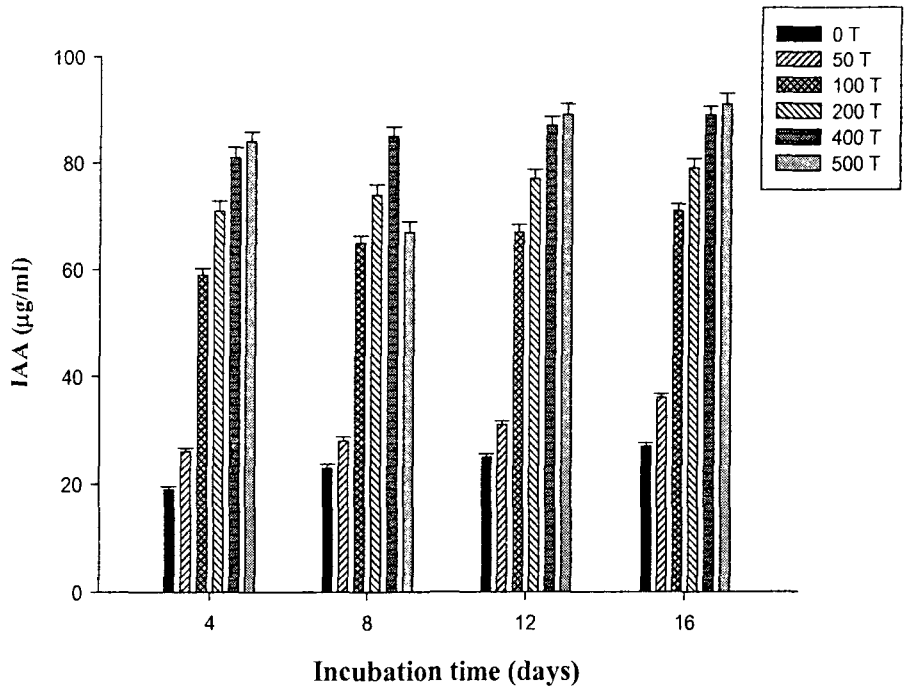

Fig. 2 8 Indole acetic acid production by *Enterobacter* strain ES2 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

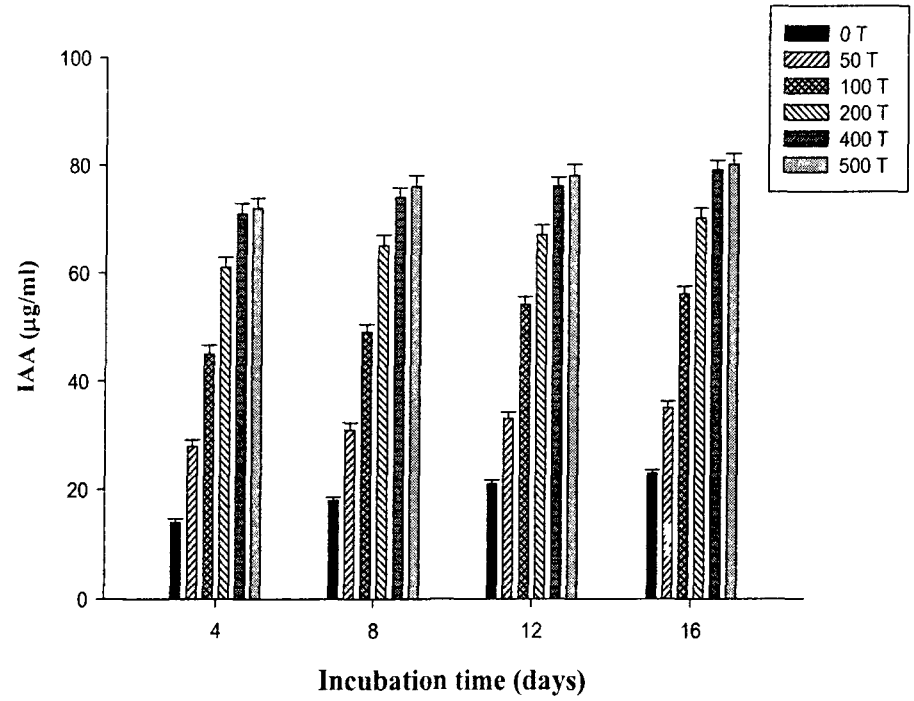

Fig. 29 - Indole acetic acid production by *B. pumilus* strain ES3 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

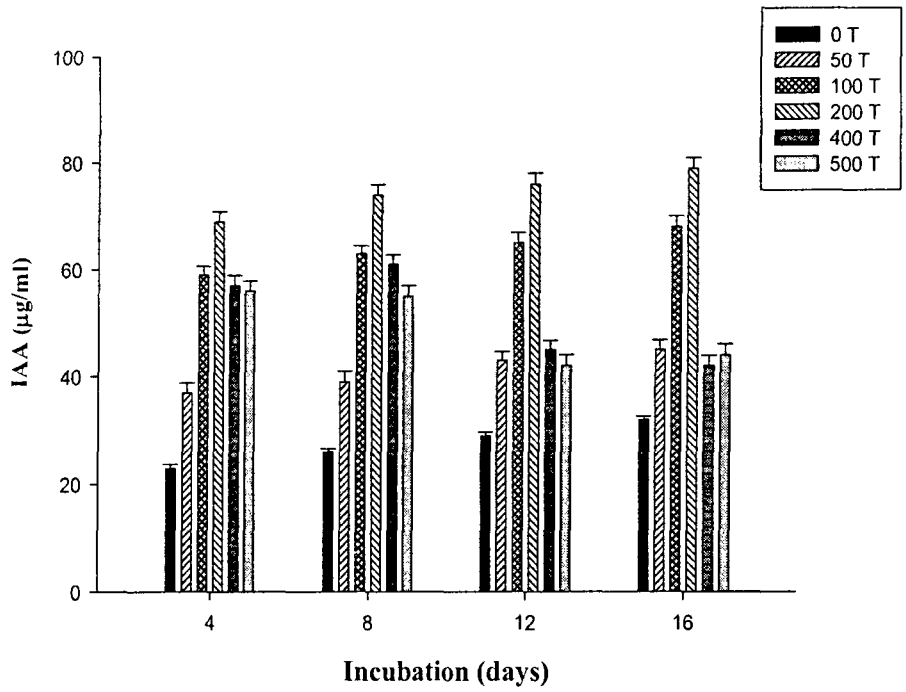

Fig. 30 Indole acetic acid production by *Pseudoxanthomonas* strain ES4 grown in Luria bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

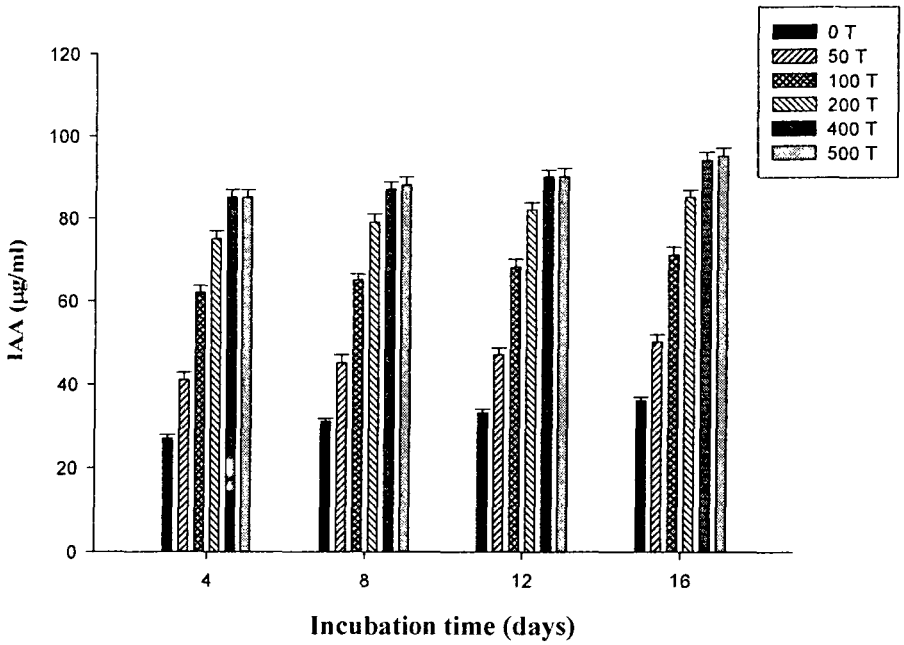

Fig. 31 Indole acetic acid production by *Stenotrophomonas* strain ES5 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

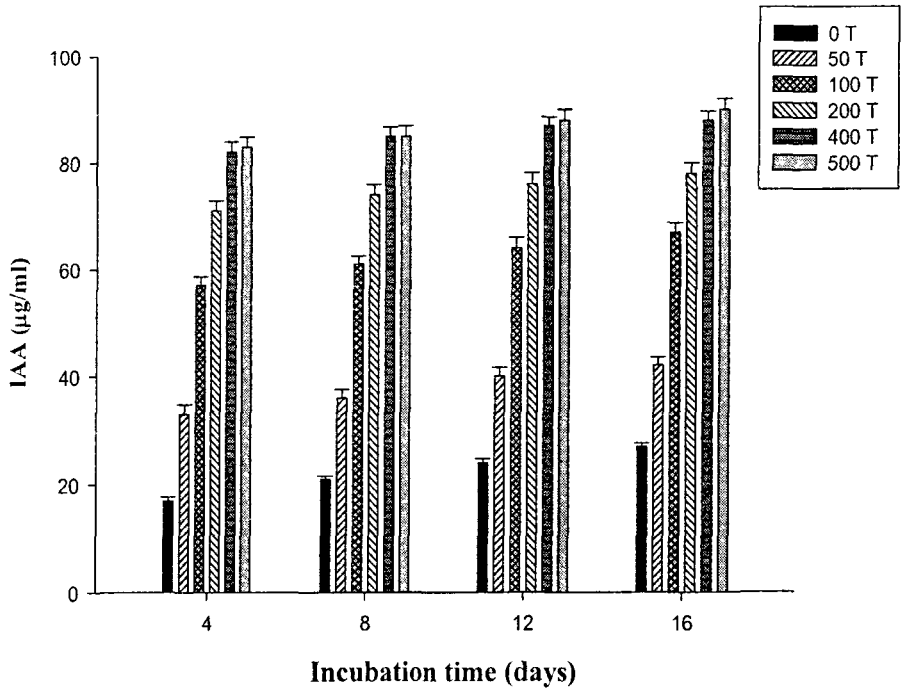

Fig. 32 Indole acetic acid production by *Achromobacter* strain ES6 grown in Luria Bertani broth supplemented with varying concentrations of tryptophan (µg/ml) at different incubation periods

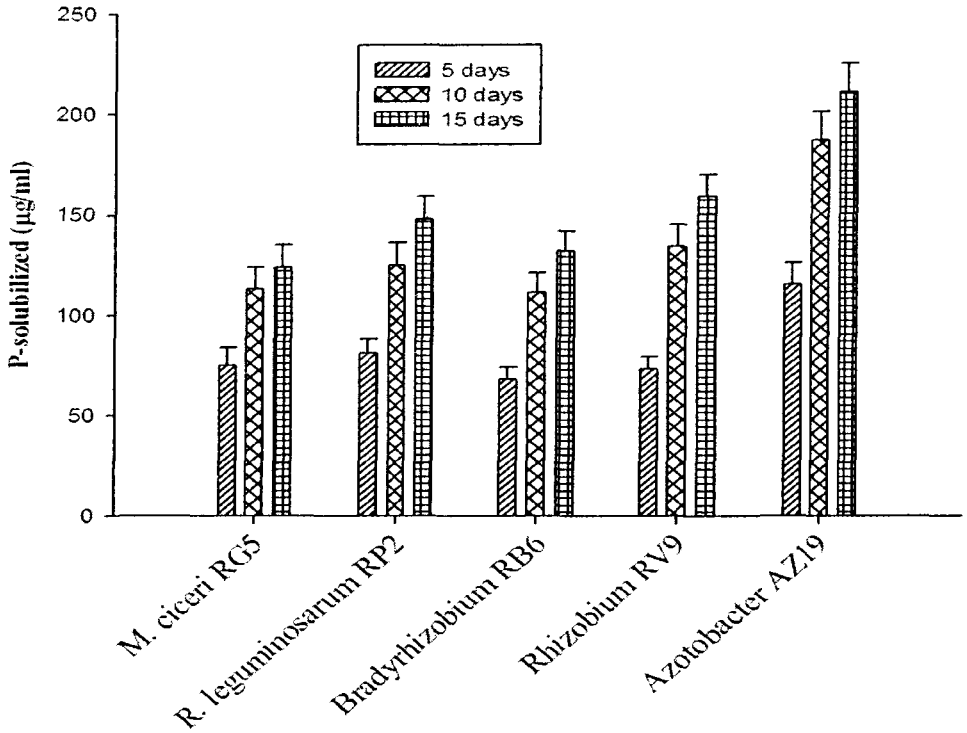

Fig. 33 Quantitative estimation of P-solubilization at different time interval in Pikovskaya broth by N-fixers

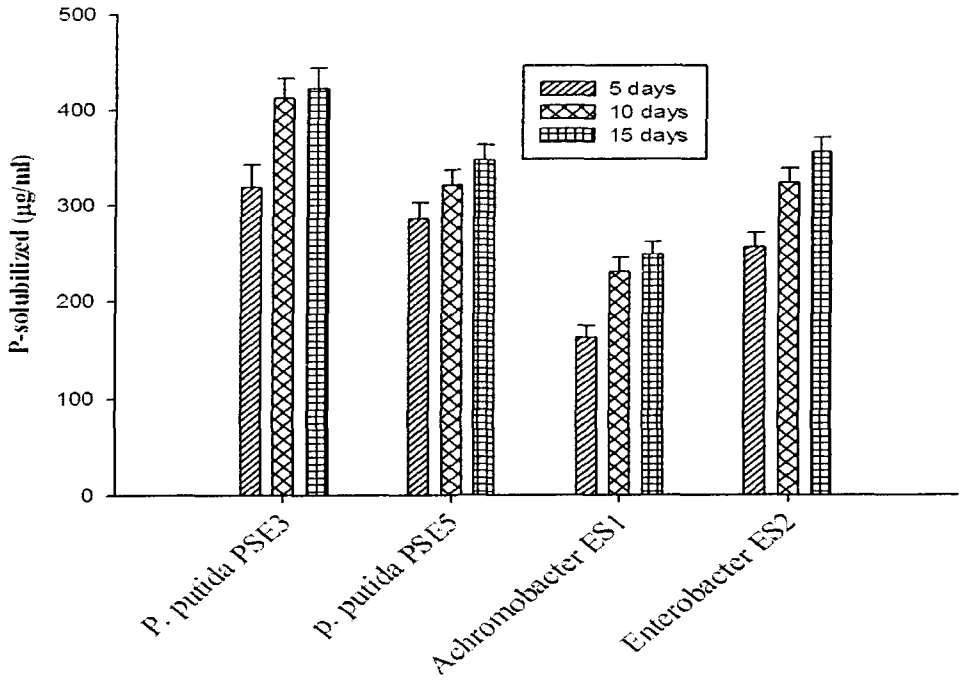

Fig. 34- Quantitative estimation of P-solubilization at different time interval in Pikovskaya broth by P-solubilizers

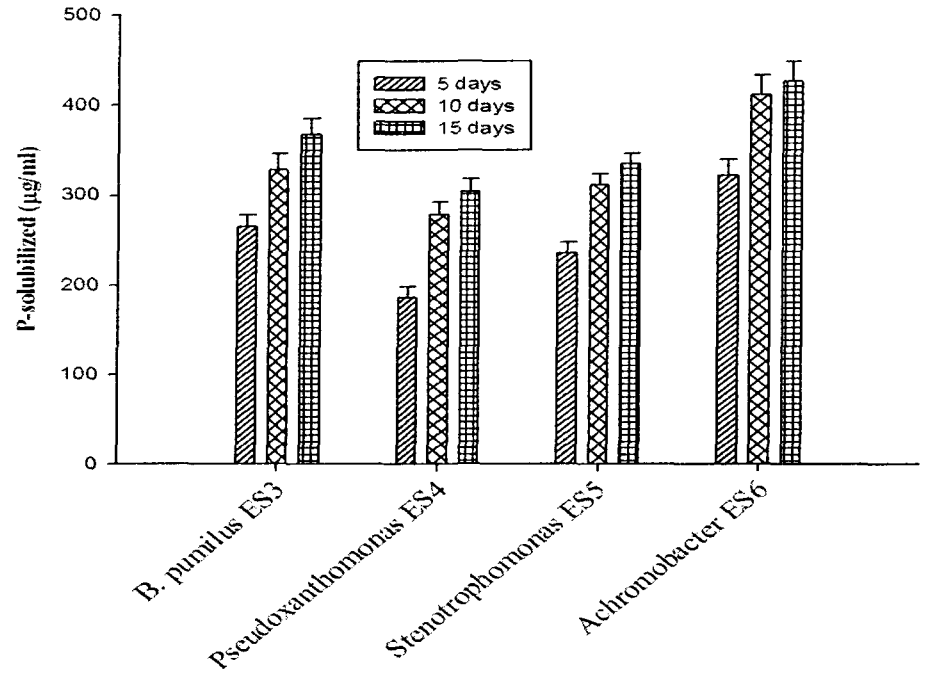

Fig. 35- Quantitative estimation of P-solubilization at different time interval in Pikovskaya broth by P-solubilizers

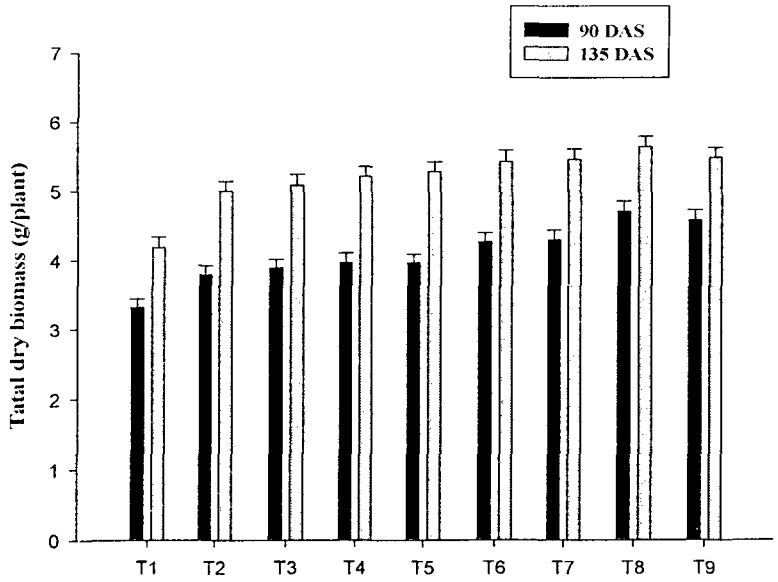

Fig. 36 Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on dry matter accumulation of chickpea plants grown in pot

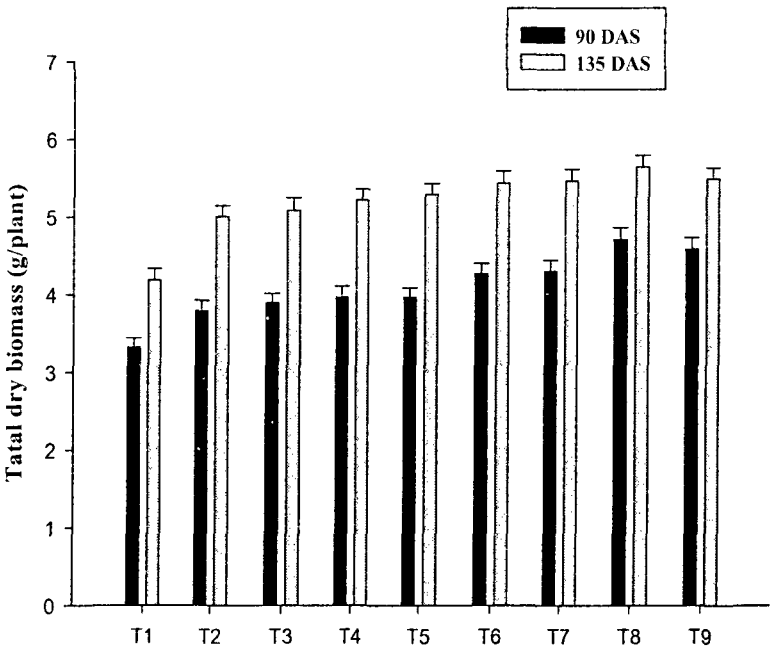

Fig. 37 Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *M. ciceri* strain RG5 on dry matter accumulation of chick pea plants grown in field conditions

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (80 kg/ha); T4-*P. putida*; T5-*M. ciceri*; T6-Urea+ *P. putida*; T7- DAP+ *M. ciceri*; T8- *P. putida*+ *M. ciceri*; T9-Urea +DAP; bar indicates the mean value of three replicates where each replicate constituted tree plants/pot or plot. Mean values are significant at $P \leq 0.05$.

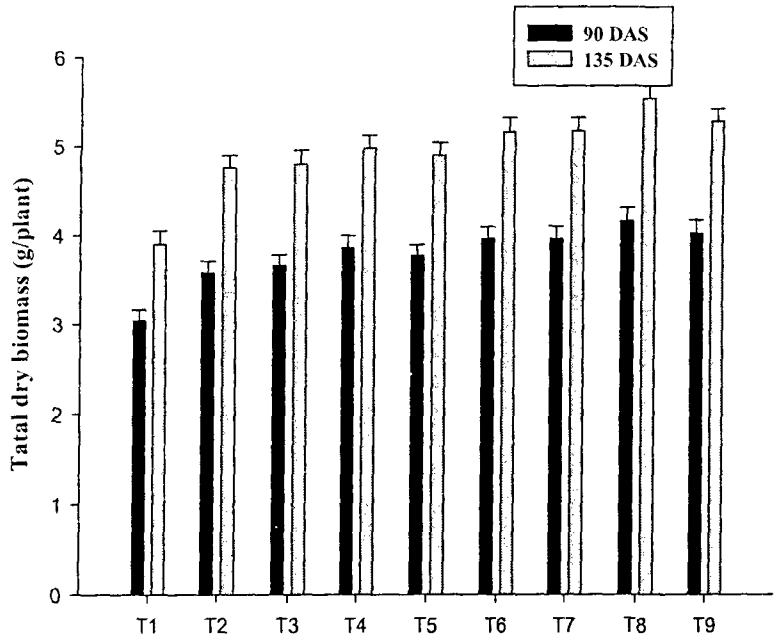

Fig. 38 Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on dry matter accumulation of chickpea plants grown in pot

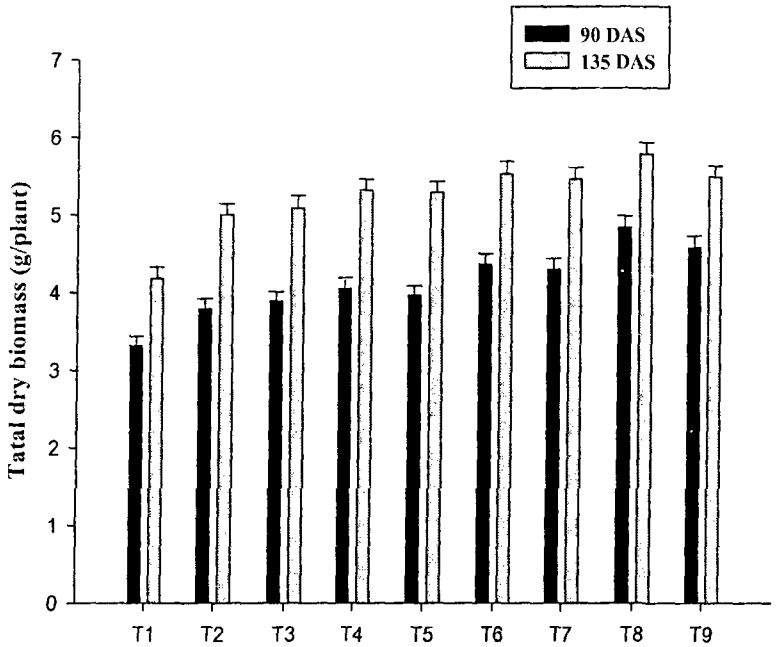

Fig. 39 Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *M. ciceri* strain RG5 on dry matter accumulation of chickpea plants grown in field conditions

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (80 kg/ha); T4-*B. pumilus* ; T5-*M. ciceri*; T6-Urea+ -*B. pumilus*; T7- DAP+ *M. ciceri*; T8- -*B. pumilus* + *M. ciceri*; T9-Urea +DAP

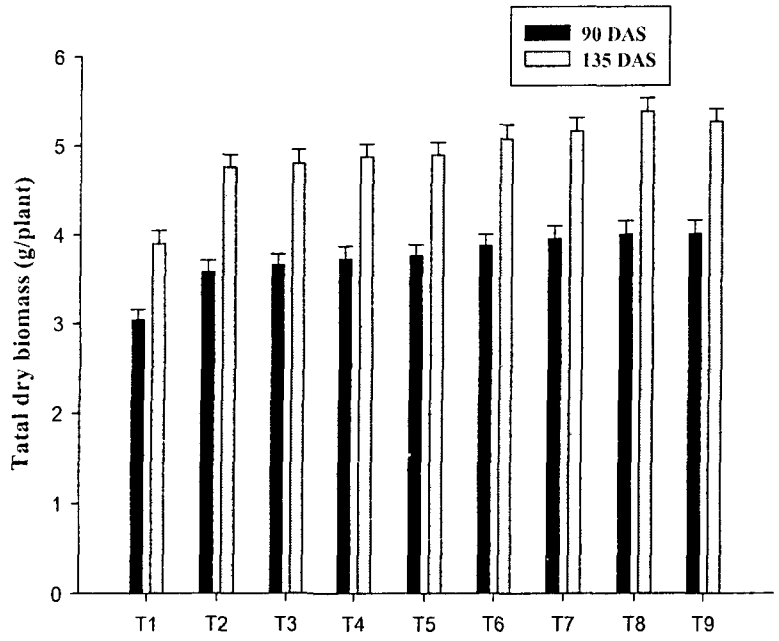

Fig. 40 Coinoculation effects of ACC deaminase producing *M. ciceri* strain RG5 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of chickpea plants grown in pot

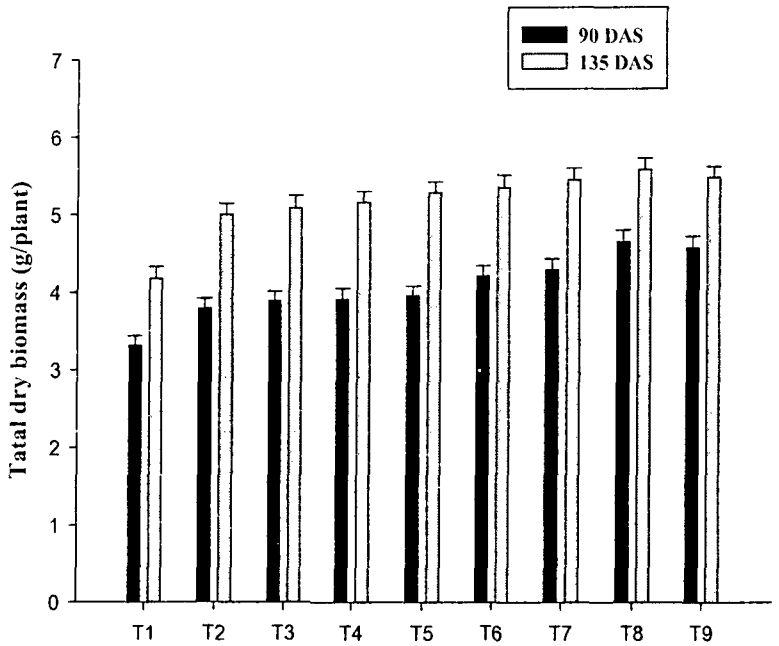

Fig. 41 Coinoculation effects of ACC deaminase producing *M. ciceri* strain RG5 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of chickpea plants grown in field

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (80 kg/ha); T4-*Azotobacter*; T5-*M. ciceri*; T6-Urea+ *Azotobacter*; T7- DAP+ *M. ciceri*; T8- *Azotobacter* + *M. ciceri*; T9-Urea +DAP

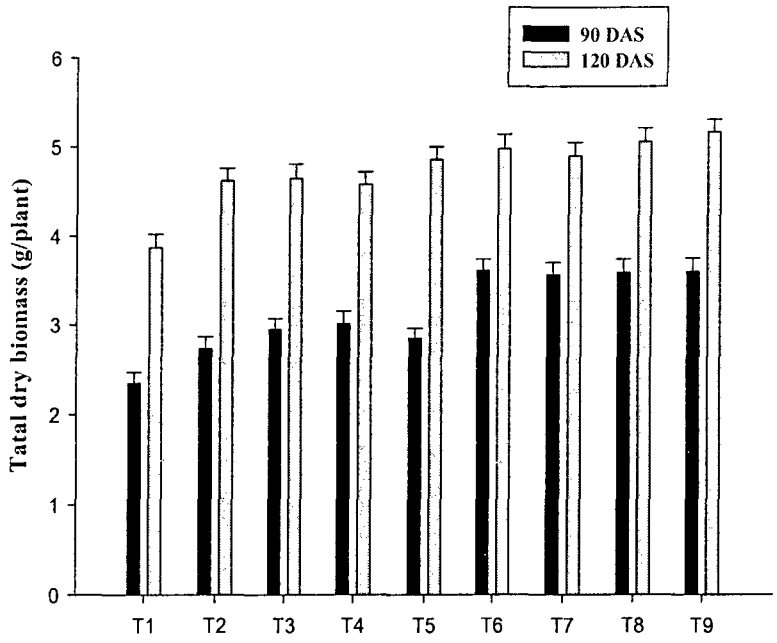

Fig. 42 Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on dry matter accumulation of pea plants grown in pot

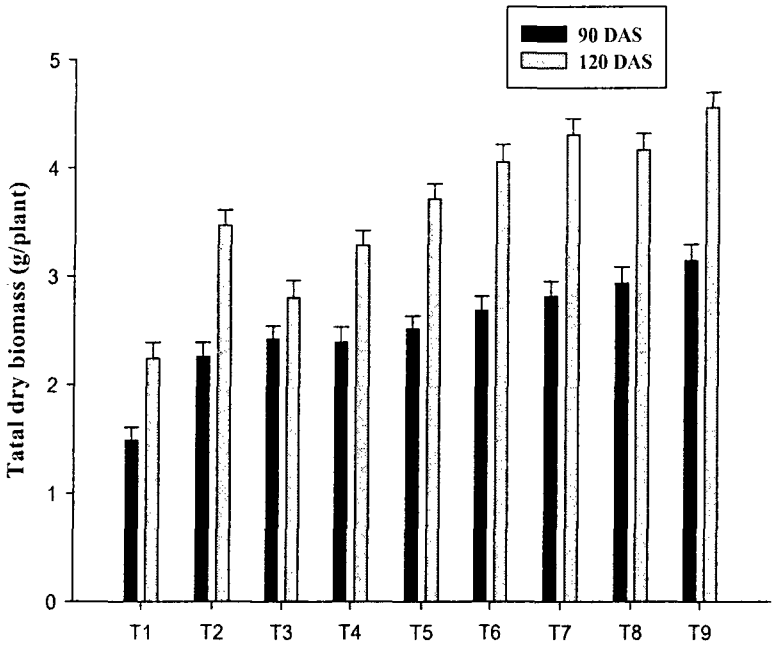

Fig. 43· Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *R. leguminosarum* strain RP2 on dry matter accumulation of pea plants grown in field

T1 indicates Control; T2-Urea (20 kg/ha); T3-DAP (90 kg/ha); T4-*P. putida*; T5-*R. leguminosarum*; T6-Urea+ *P. putida*; T7- DAP+ *R. leguminosarum*; T8- *P. putida*+ *R. leguminosarum*; T9-Urea +DAP

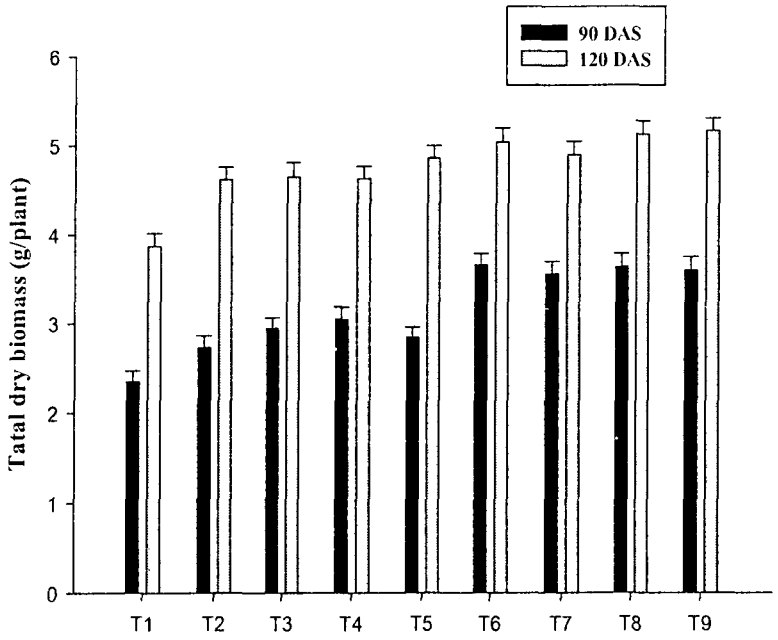

Fig. 44 Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on dry matter accumulation of pea plants grown in pot

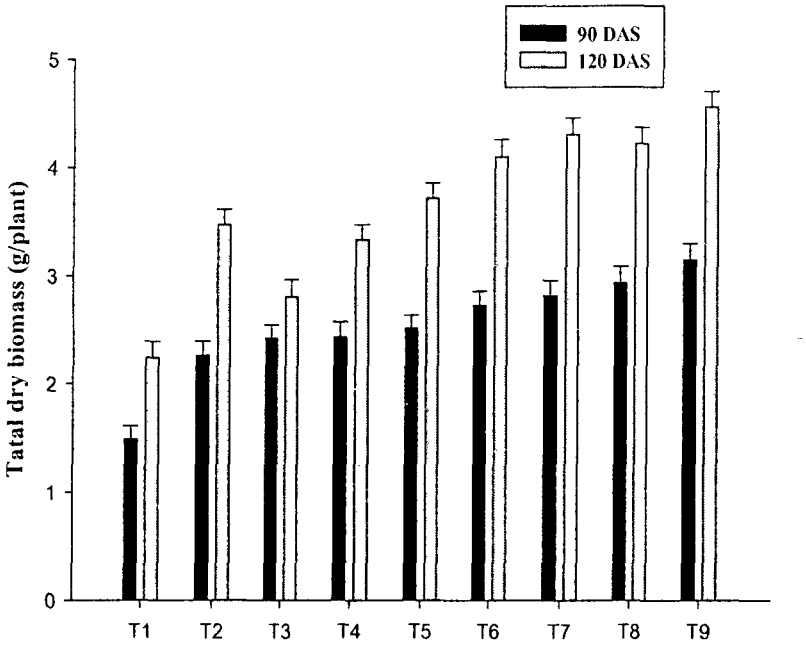

Fig. 45 Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *R. leguminosarum* strain RP2 on dry matter accumulation of pea plants grown in field

T1 indicates Control; T2-Urea (20 kg/ha); T3-DAP (90 kg/ha); T4-*B. pumilus*; T5-*R. leguminosarum*; T6-Urea+ *B. pumilus*; T7- DAP+ *R. leguminosarum*; T8- *B. pumilus* + *R. leguminosarum*; T9-Urea +DAP

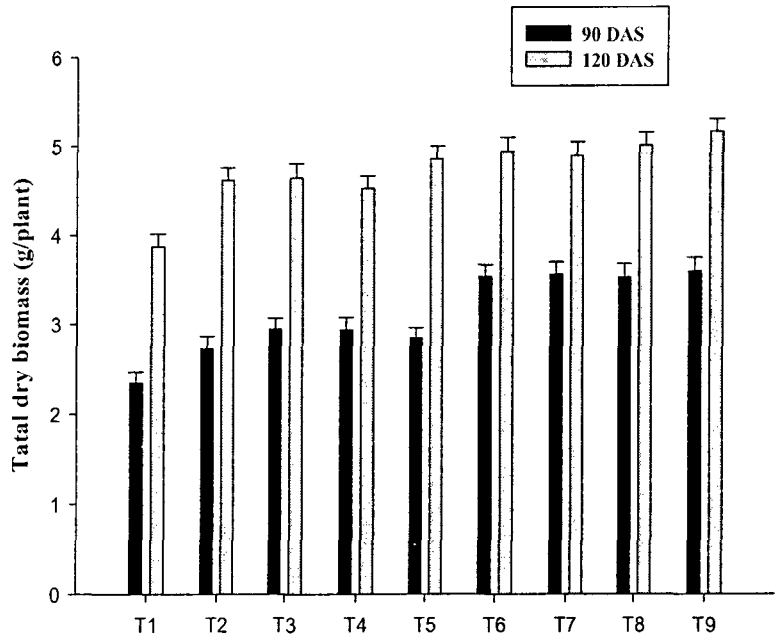

Fig. 46- Coinoculation effects of ACC deaminase producing *R. leguminosarum* strain RP2 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of pea plants grown in pot

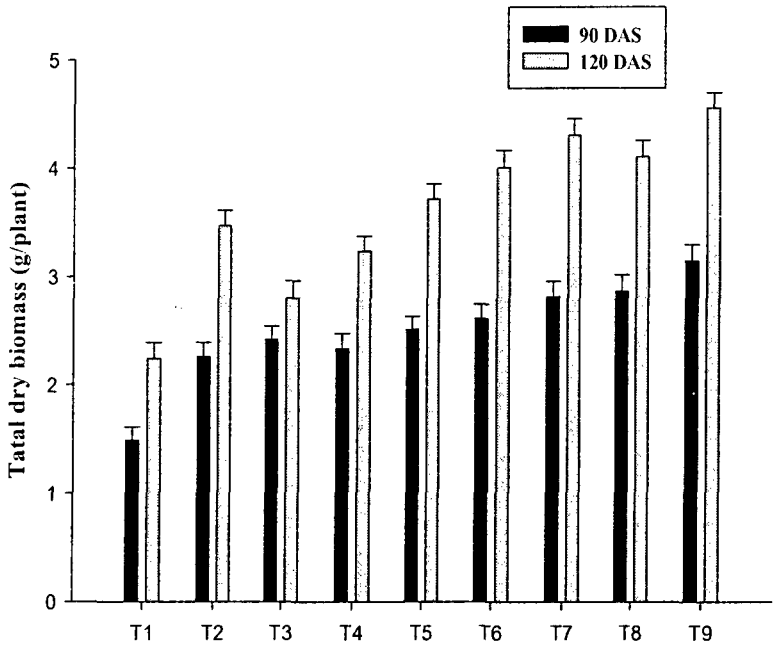

Fig. 47- Coinoculation effects of ACC deaminase producing *R. leguminosarum* strain RP2 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of pea plants grown in field

T1 indicates Control; T2-Urea (20 kg/ha); T3-DAP (90 kg/ha); T4-*Azotobacter*; T5-*R. leguminosarum*; T6-Urea+ *Azotobacter*; T7- DAP+ *R. leguminosarum*; T8-*Azotobacter* + *R. leguminosarum*; T9-Urea +DAP

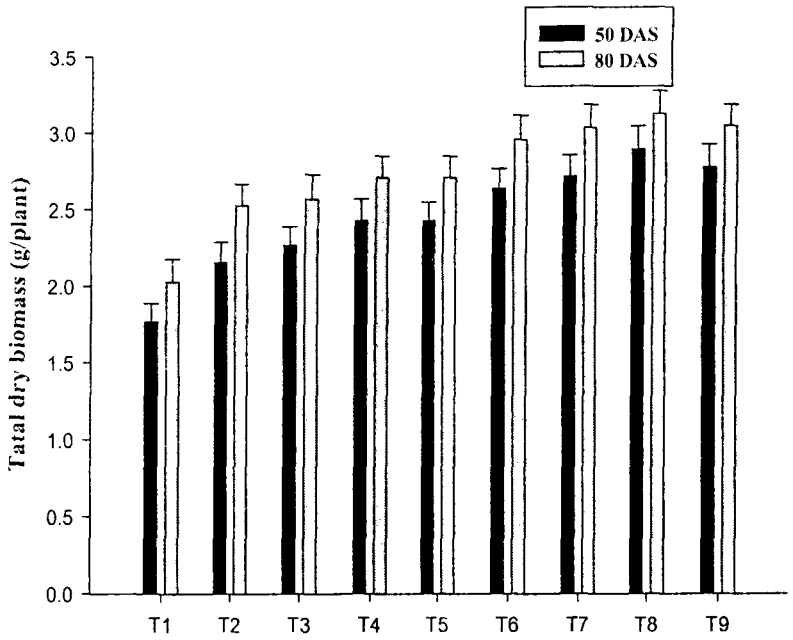

Fig. 48 Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on dry matter accumulation of greengram plants grown in pot

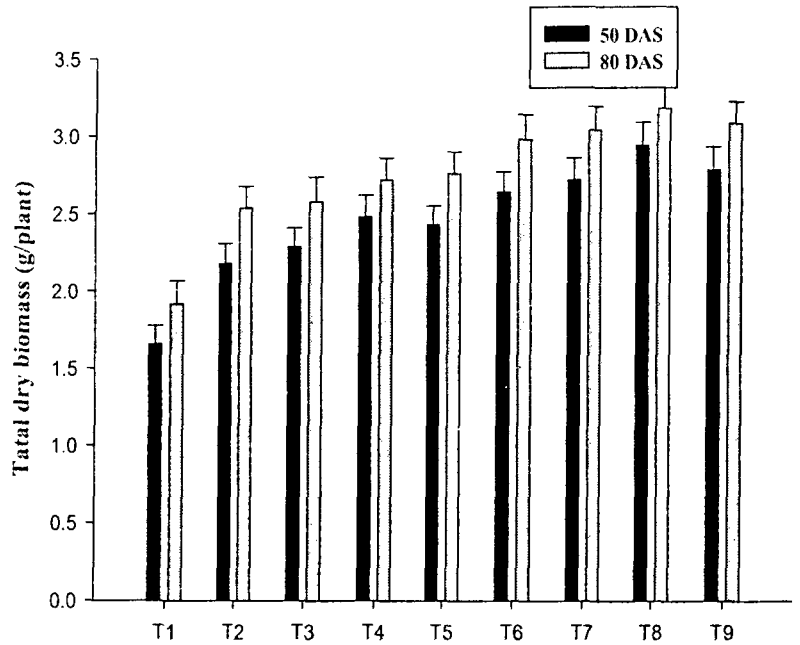

Fig. 49 - Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Bradyrhizobium* strain RB6 on dry matter accumulation of greengram plants grown in field

T1 indicates Control; T2-Urea (25 kg/ha); T3-DAP (85 kg/ha); T4-*P. putida*; T5-*Bradyrhizobium*; T6-Urea+ *P. putida*; T7- DAP+ *Bradyrhizobium*; T8- *P. putida*+ *Bradyrhizobium*; T9-Urea +DAP

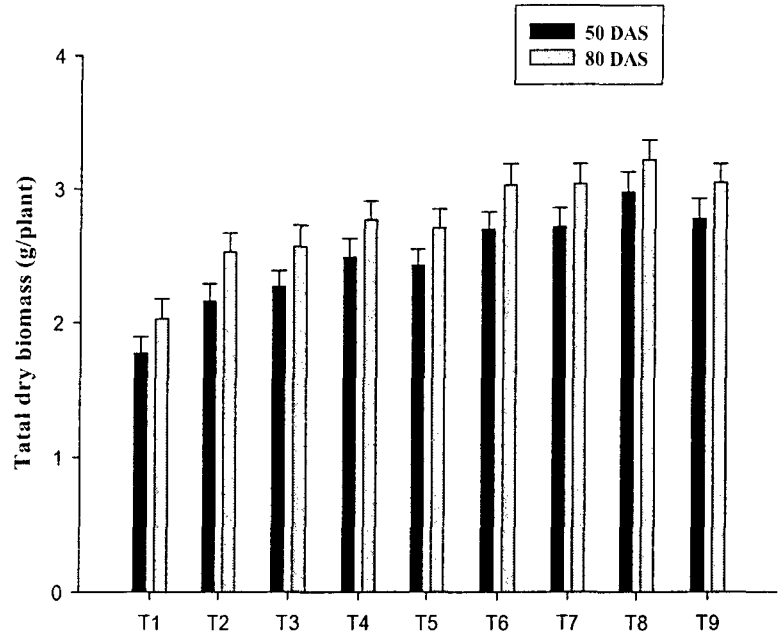

Fig. 50- Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on dry matter accumulation of greengram plants grown in pot

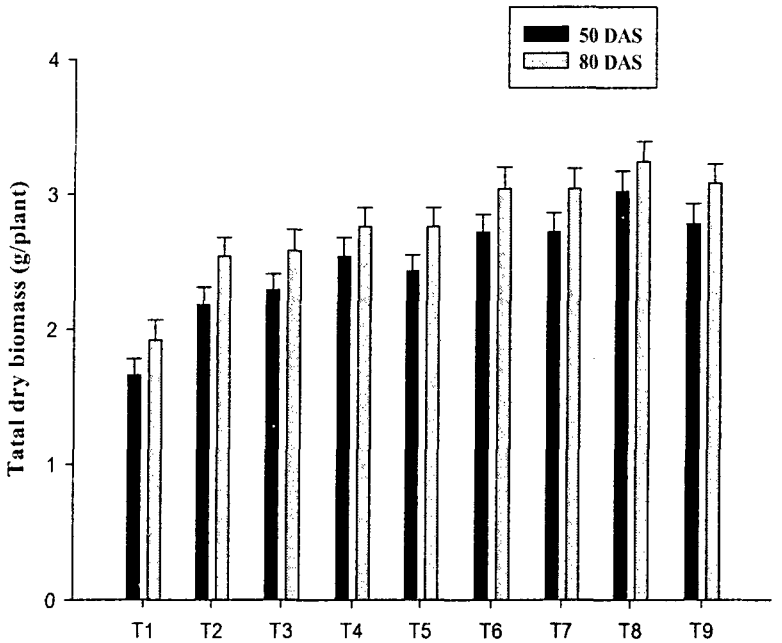

Fig. 51- Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Bradyrhizobium* strain RB6 on dry matter accumulation of greengram plants grown in field

T1 indicates Control; T2-Urea (25 kg/ha); T3-DAP (85 kg/ha); T4-*B. pumilus*; T5-*Bradyrhizobium*; T6-Urea+ -*B. pumilus*; T7- DAP+ *Bradyrhizobium*; T8- -*B. pumilus* + *Bradyrhizobium*; T9-Urea +DAP

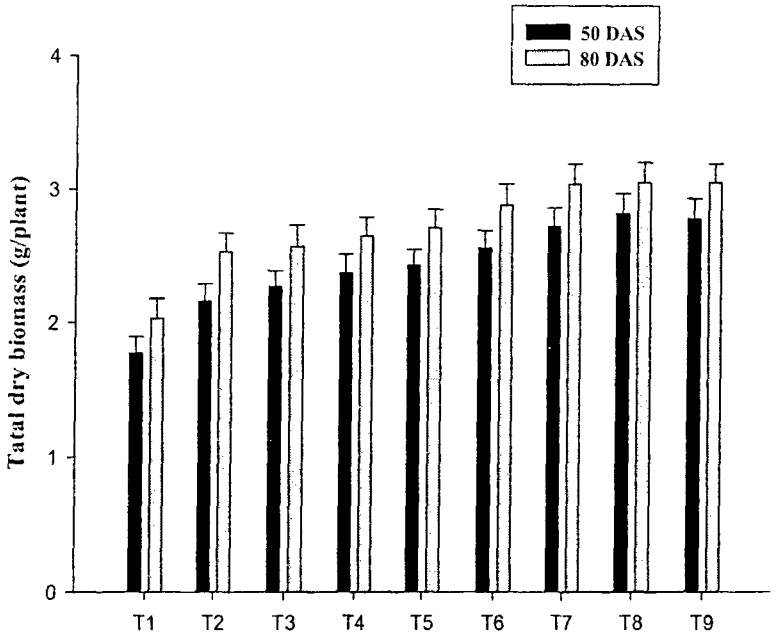

Fig. 52- Coinoculation effects of ACC deaminase producing *Bradyrhizobium* strain RG6 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of greengram plants grown in pot

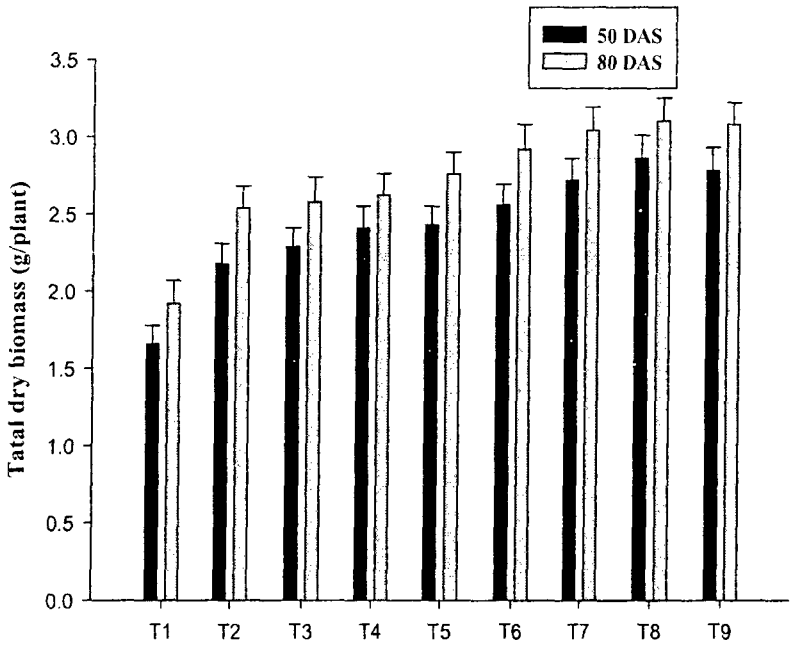

Fig. 53- Coinoculation effects of ACC deaminase producing *Bradyrhizobium* strain RG6 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of greengram plants grown in field

T1 indicates Control; T2-Urea (25 kg/ha); T3-DAP (85 kg/ha); T4-*Azotobacter*; T5-*Bradyrhizobium*; T6-Urea+ *Azotobacte*; T7- DAP+ *Bradyrhizobium*; T8- *Azotobacte*+ *Bradyrhizobium*; T9-Urea +DAP

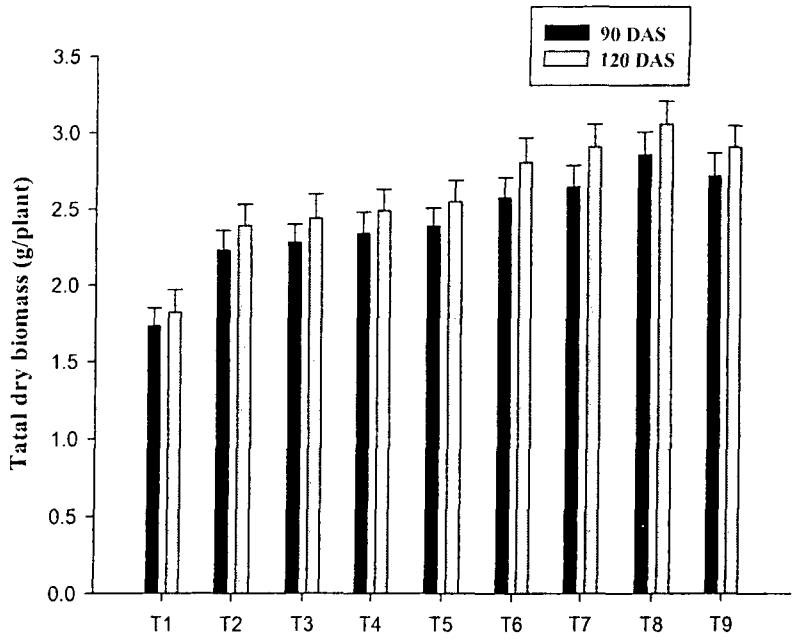

Fig. 54- Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on dry matter accumulation of lentil plants grown in pot

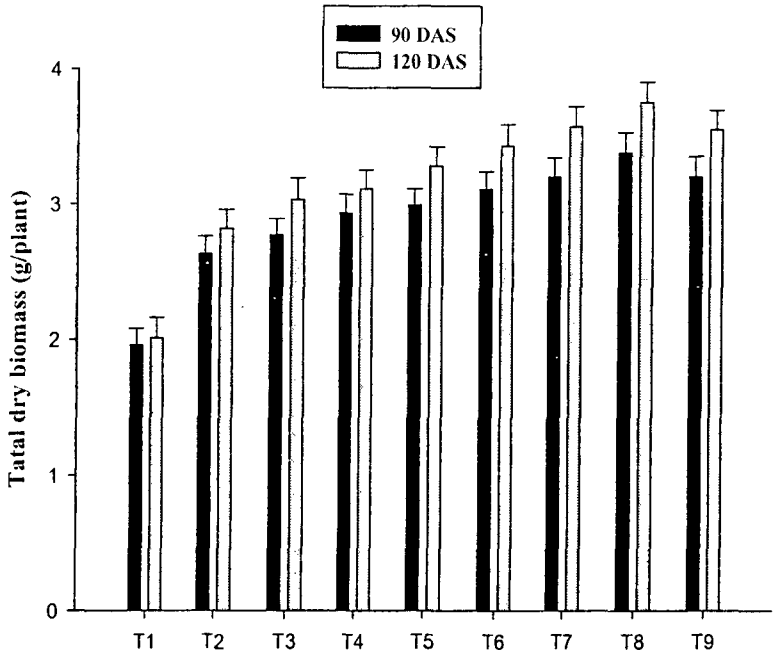

Fig. 55- Coinoculation effects of ACC deaminase producing *P. putida* strain PSE3 and *Rhizobium* strain RV9 on dry matter accumulation of lentil plants grown in field

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (90 kg/ha); T4-*P. putida*; T5-*Rhizobium*; T6-Urea+ *P. putida*; T7- DAP+ *Rhizobium*; T8- *P. putida*+ *Rhizobium*; T9-Urea +DAP

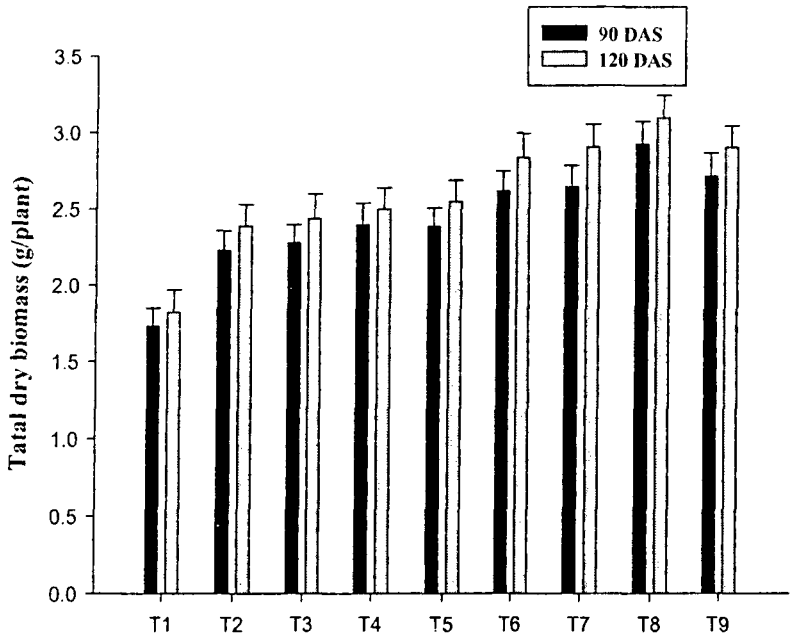

Fig. 56- Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on dry matter accumulation of lentil plants grown in pot

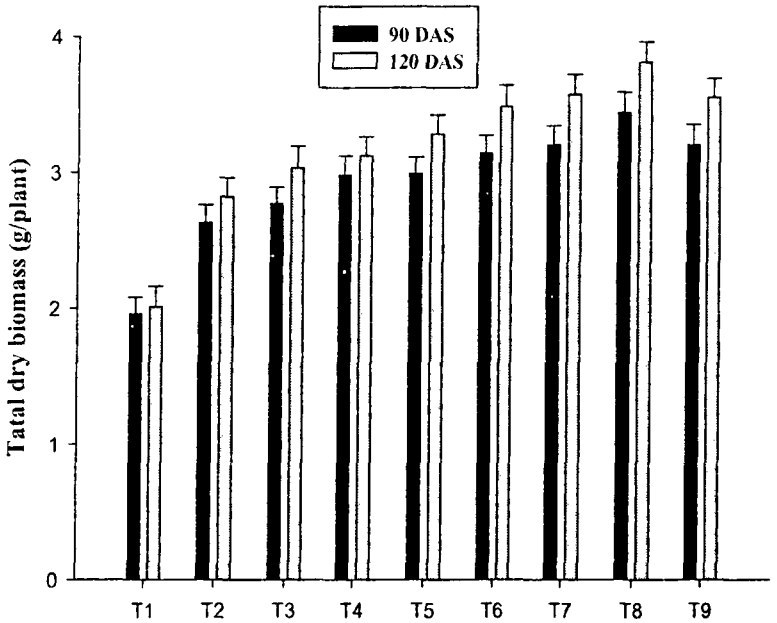

Fig. 57- Coinoculation effects of ACC deaminase producing *B. pumilus* strain ES3 and *Rhizobium* strain RV9 on dry matter accumulation of lentil plants grown in field

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (90 kg/ha); T4-*B. pumilus*; T5-*Rhizobium*; T6-Urea+ *B. pumilus*; T7- DAP+ *Rhizobium*; T8- *B. pumilus* + *Rhizobium*; T9-Urea +DAP

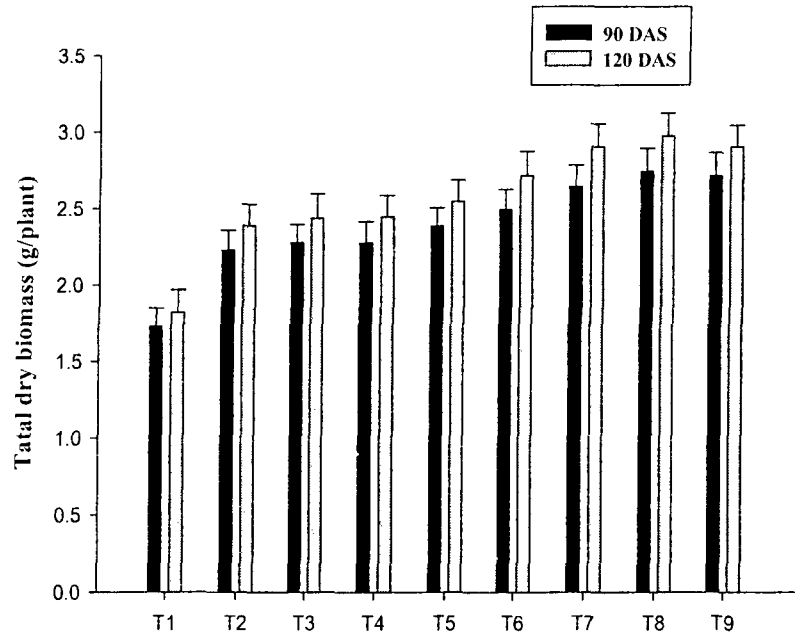

Fig. 58- Coinoculation effects of ACC deaminase producing *Rhizobium* strain RV9 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of lentil plants grown in pot

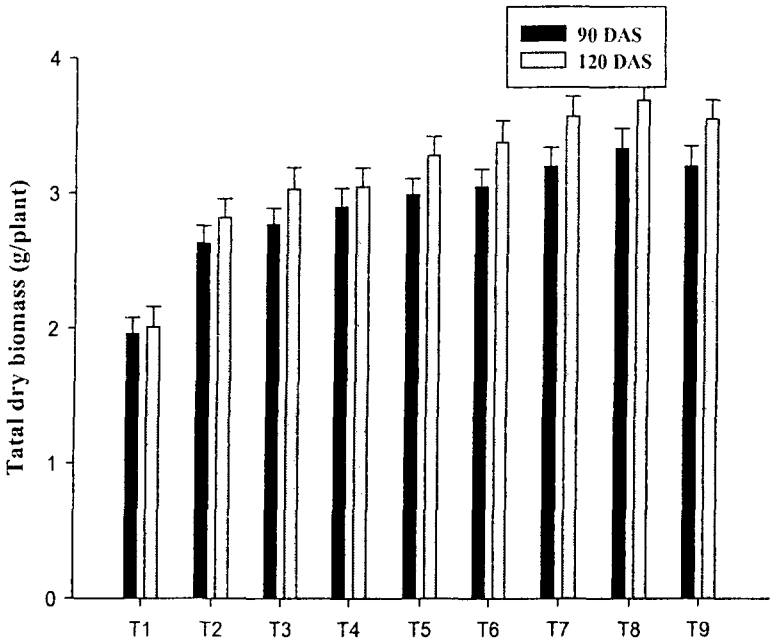

Fig. 59- Coinoculation effects of ACC deaminase producing *Rhizobium* strain RV9 and P-solubilizing *Azotobacter* strain AZ19 on dry matter accumulation of lentil plants grown in field

T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (90 kg/ha); T4-*Azotobacter*; T5-*Rhizobium*; T6-Urea+ *Azotobacter*; T7- DAP+ *Rhizobium*; T8- *Azotobacter* + *Rhizobium*; T9-Urea +DAP

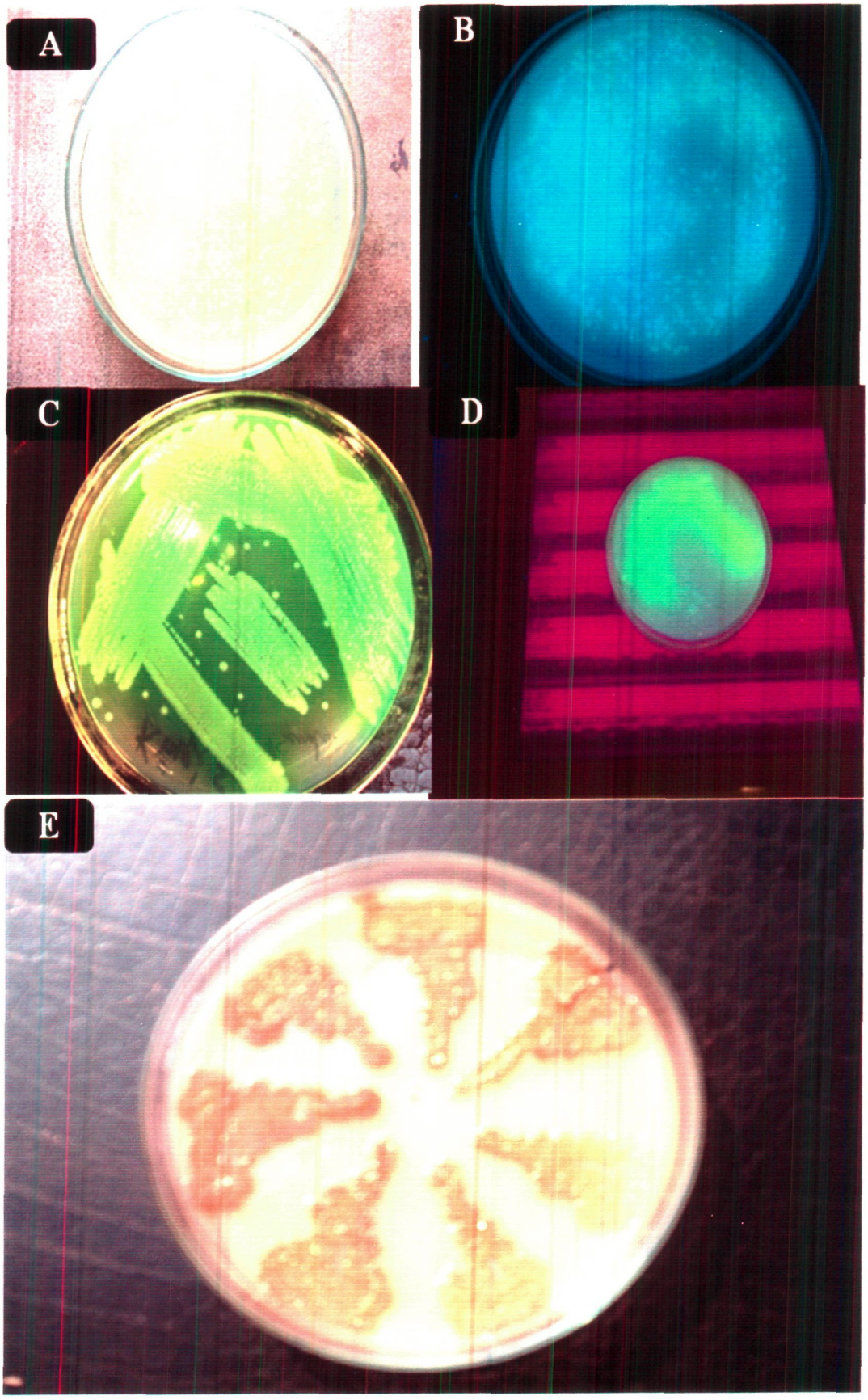

Plate 1- Functional diversity of rhizobacteria (A) *Bacillus* (B and D) Pseudomonas (E) *Azotobacter*

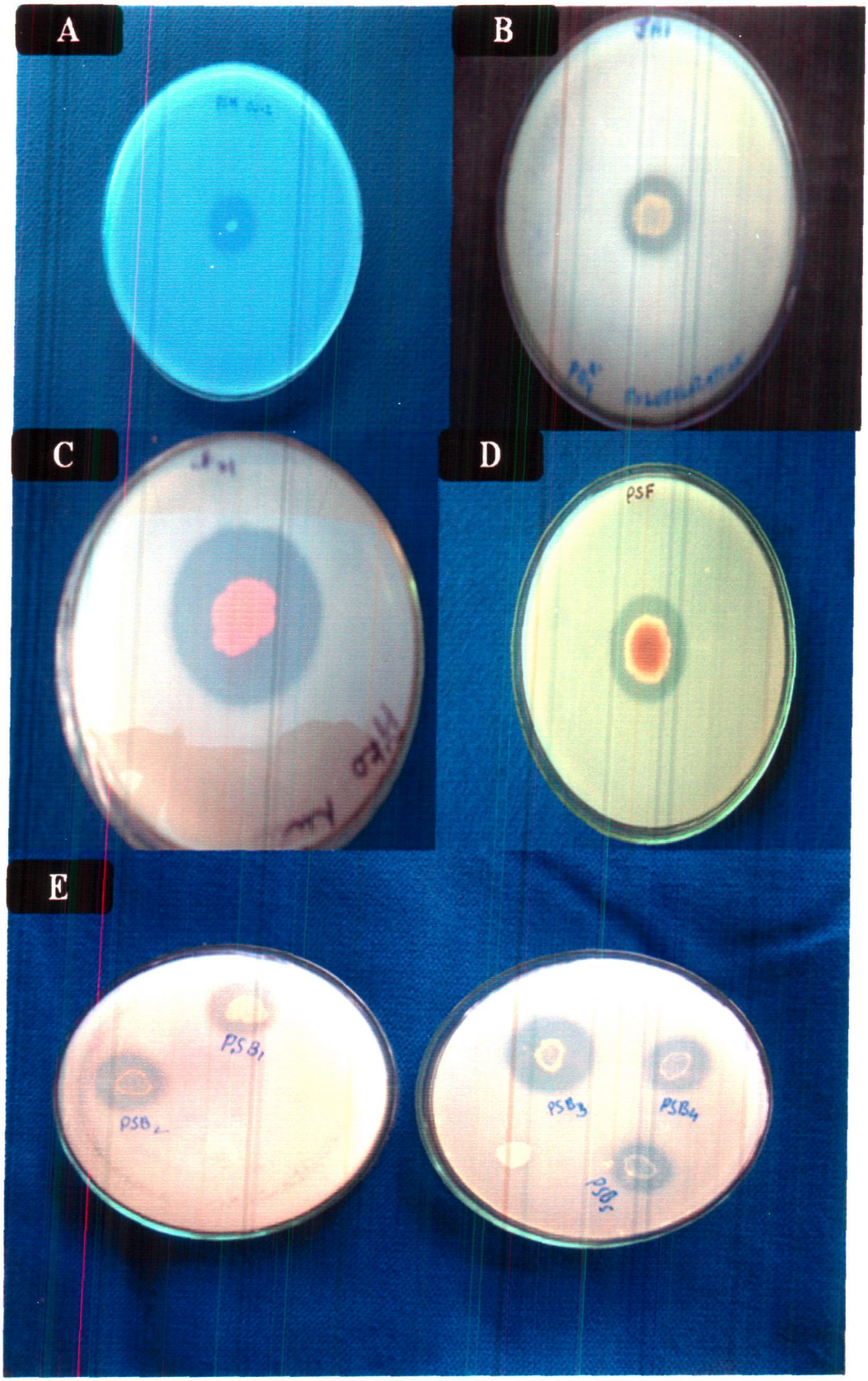

Plate 2- Phosphate solubilizers shows P-solubilization on Pikovskaya plate (A) P-solubilizer (B) *Bacillus* (B) *Azotobacter* (C) *Searratia* (D) Fungi (E) *Pseudomonas* sp.

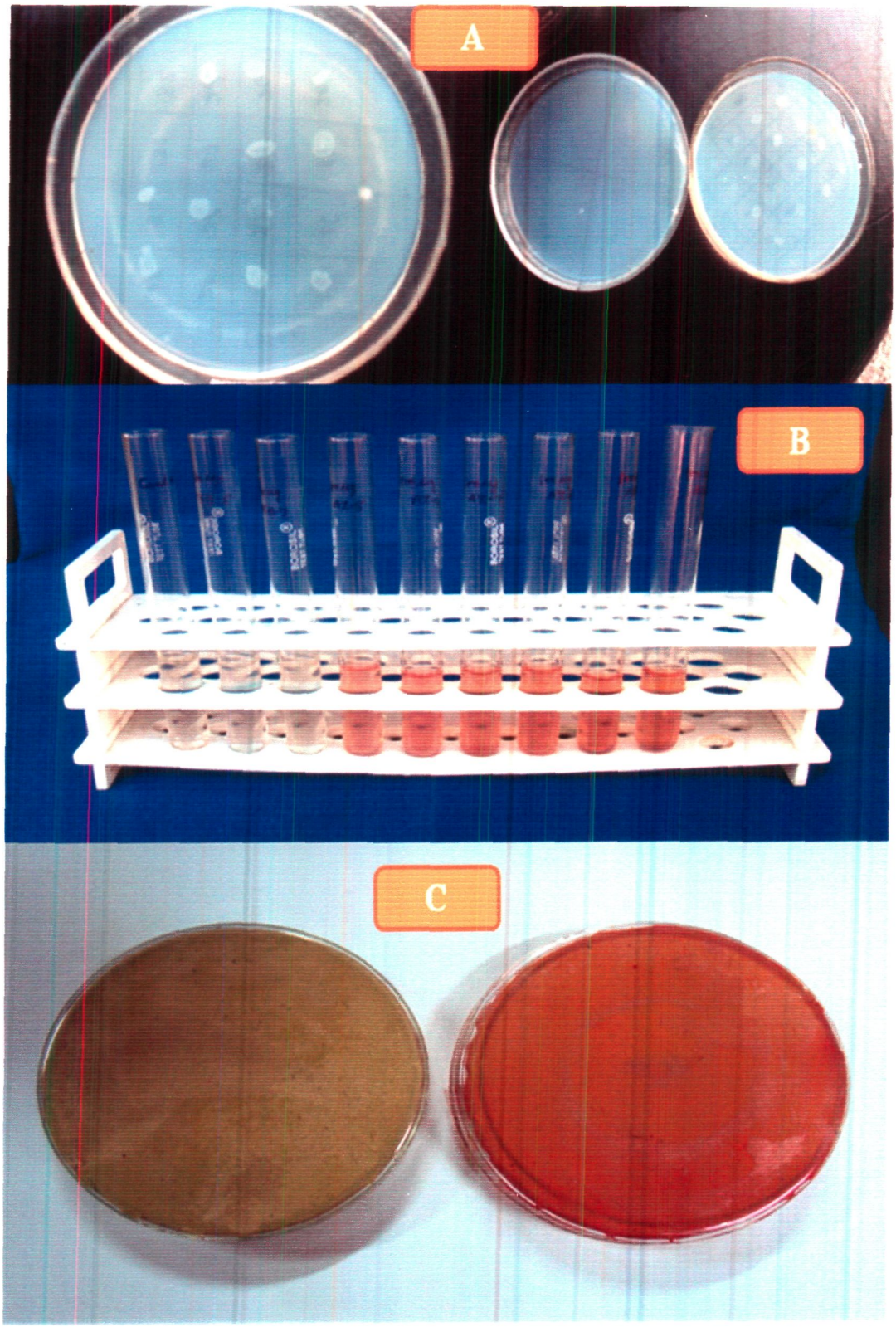

Plate 3- Plant growth promoting activity (A) ACC deaminase positive strains on DF salt medium (B) IAA production (C) HCN production

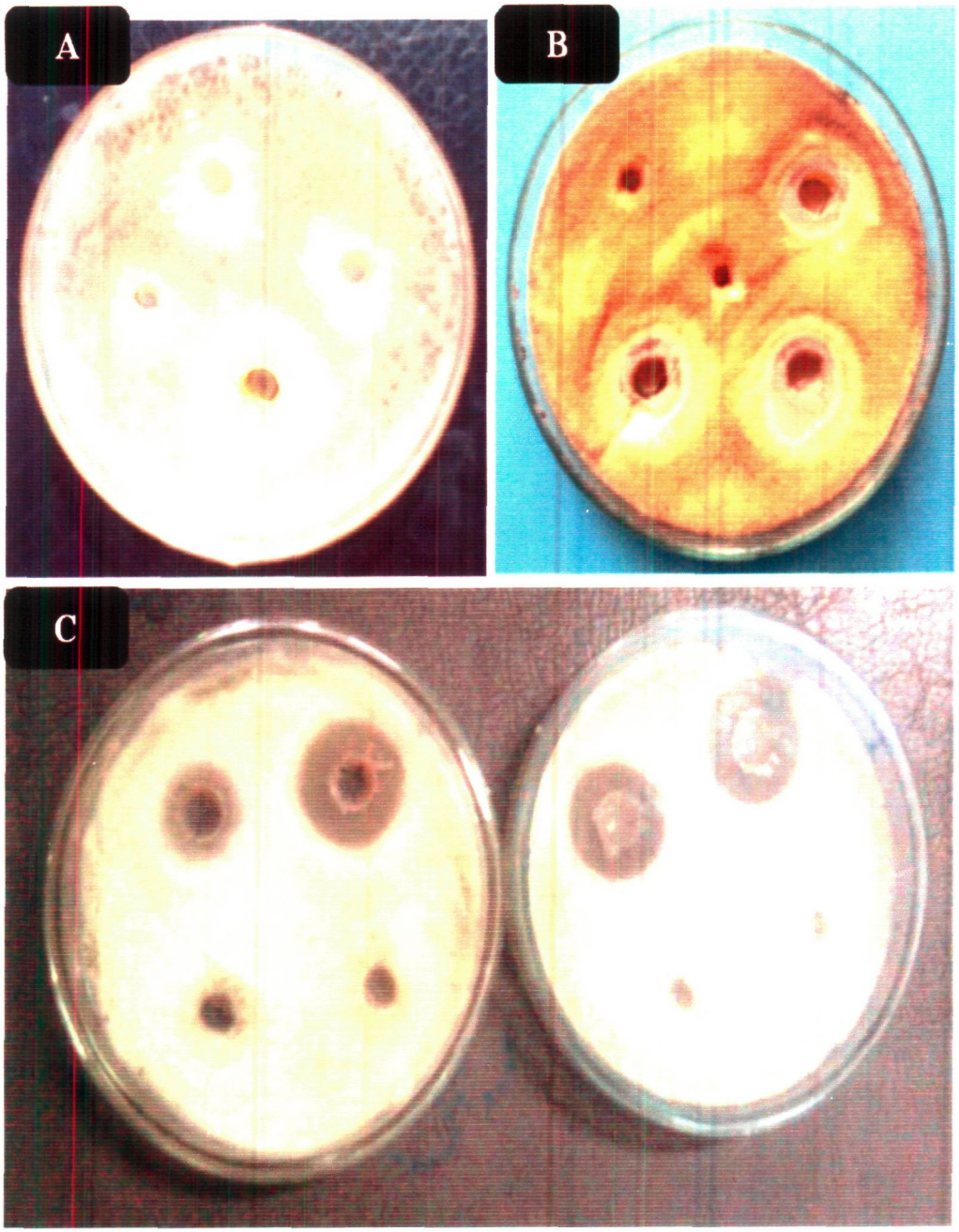

Plate 4- Antifungal activity shown by PGPR (A) *Alternaria* sp. (B) *Rhizoctonia* sp. (C) *Penicillium* sp.

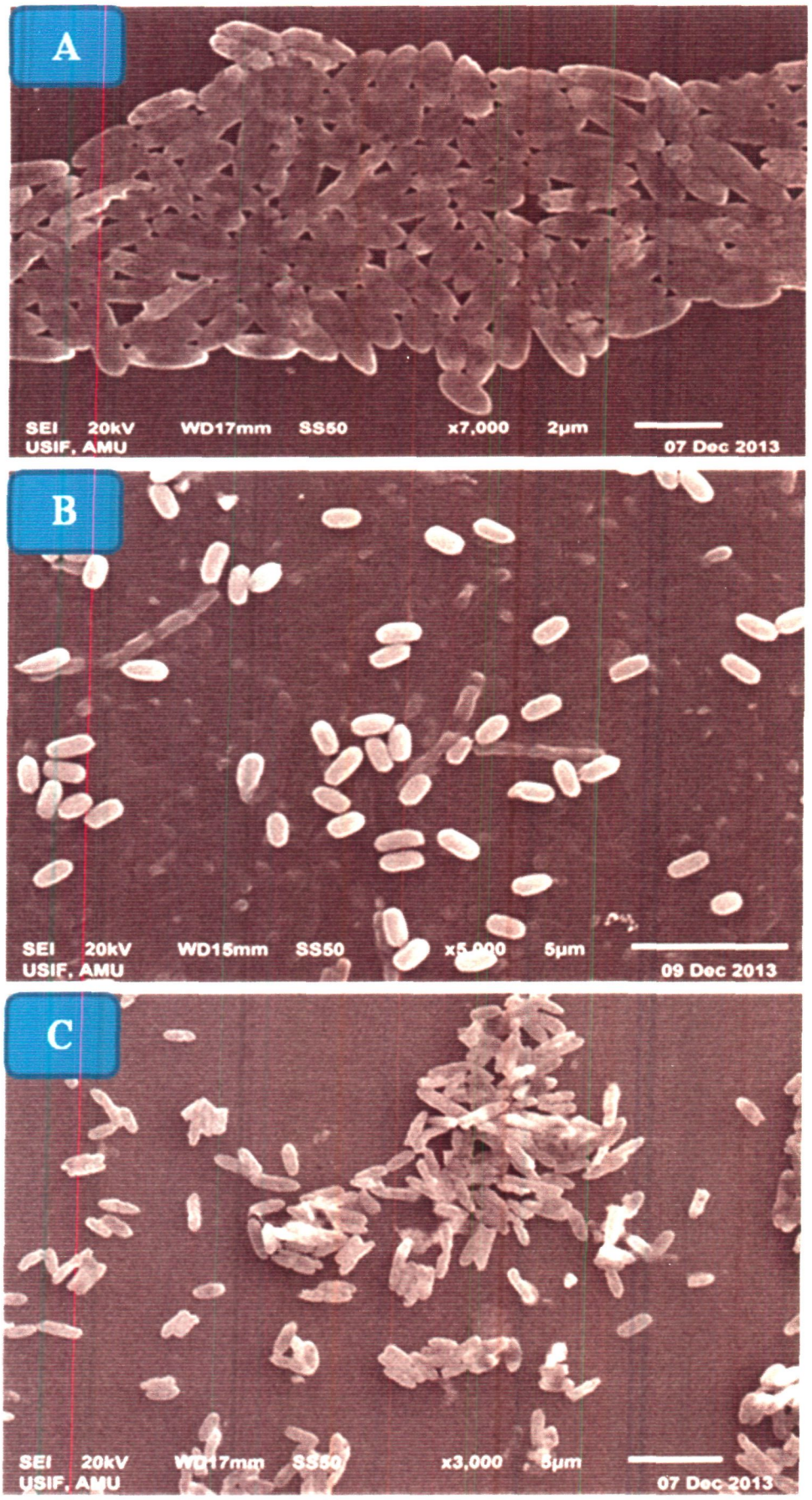

Plate 5-Scanning electron microscopy of bacterial strains (A) *P. putida* strain PSE3 (B) *B. pumilus* strain ES3 (C) *Azotobacter* strain AZ19

Plate 6- Effect of inoculation and fertilizer on greengram [T1 indicates Control; T2-Urea (30 kg/ha); T3-DAP (80 kg/ha); T4-*B. pumilus* ; T5-*M. ciceri*; T6-Urea+ -*B. pumilus*; T7- DAP+ *M. ciceri*; T8- -*B. pumilus* + *M. ciceri*; T9-Urea +DAP]

Plate 8- Effect of inoculation and fertilizer on chickpea plants in field trials

Plate 9- Effect of inoculation and fertilizer on pea plants in field trials

Plate 10- Nodulation on chickpea plant

5.1 Microbial diversity in rhizospheric soils of legume and non-legume crops

Microbial populations inhabiting different rhizospheric/non-rhizospheric soils (Steudel et al., 2012) across different production systems do play some major roles in influencing soil fertility by involving in numerous biogeochemical cycles. Also, the heterogeneously distributed microbial communities within soils affects the above-ground ecosystems by supplying essential nutrients to plants (Dodd and Ruiz-Lozano 2012), improve soil structures and ultimately, influence soil nutrient pool (Kirk et al., 2004; Gholami et al., 2009). Apart from such important activities, soil microflora also affects other soil process for example nutrient mobilization and mineralization (Adesemoye et al., 2008), degradation of complex compounds (Degelmann et al., 2009), release of nutrients (Ponmurugan and Gopi 2006), phosphate solubilization (Kumar et al., 2008; Khan et al., 2009), denitrification (Kim et al., 2008), N_2 fixation (Hara et al., 2009), and suppression of soil borne phytopathogens (Rameshkumar and Nair 2009). Due to such vast interactive activities of microflora occurring in soils, the microbial diversity within different soil ecosystems changes over time. In a study, Torsvik et al., (1997) for example reported a rapid decline in microbial diversity in perturbed soil due to agriculture, as compared to conventional environments. In addition, the microbial diversity changes alarmingly in varied soils ecosystem, or it may vary from plant genotype type to genotype. Hence, it becomes utterly important to better understand the structural and functional variation among microbes inhabiting soil niches (Nannipieri 1994, Tarafdar and Claassen 1988, Dilly and Munch 1998). In order to unravel the true characteristics of microbial functionality, microbiologists especially those involved in sustainable and more practical agronomic practices are desperate to find and assess the impact of farm practices/process, or disturbance on the activity or composition of the soil microbial community. Considering the vast and varied functional aspects of microbial communities and their consequential impact on crop production across different climatic regions, the present study was designed to look into the finer details of such activities exhibited by microflora.

During this study, the microbial diversity among different rhizospheric soils of mentha, chilli, cabbage, mustard, chickpea, pea, greengram and lentil plants grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, India was assayed. A significant variation in microbial composition of rhizospheric soils of legumes and other non-legumes was observed. Generally, the microbial populations (mean value 3.8×10^7 cfu/g soil) that included total bacterial counts (mean value

3.7×10^7 cfu/g soil), total fungal (mean value 1.4×10^5 cfu/g soil) and actinomycetal (mean value 2.2×10^4 cfu/g soil) populations, phosphate solubilizing (PS) microorganisms involving PS bacteria (mean value 5.2×10^5 cfu/g soil) and PS fungi (mean value 5.2×10^3 cfu/g soil) and asymbiotic nitrogen fixer for example *Azotobacter* species (mean value 2.6×10^5 cfu/g soil) were considerably higher in the soil samples collected from different rhizospheres than did the non rhizospheric soil samples (mean value 2.5×10^7 cfu/g soil). While comparing the microbial diversity in different rhizospheres, mentha rhizosphere in general was found to have highest total microbial populations (mean value 4.3×10^7 cfu/g soil) which were followed by greengram rhizosphere (mean value 4.2×10^7 cfu/g soil) compared to other soil samples. Among all microbial populations recovered from eight different rhizospheres, the actinomycetal population was greatly lower (2.2×10^4 cfu/g soil). Moreover, the populations of phosphate solubilizing microorganisms (mean value 5.3×10^5 cfu/g soil) including PSB (mean value $5.24\text{x}10^5$ cfu/g soil) and PS fungi (mean $5.2\text{x}10^3$ cfu/g) in all soil samples were higher. Among rhizospheres, greengram soils in general had the greatest PSM populations (7.2×10^5 cfu/g soil) than other rhizospheric soil samples. The significant variation in heterogeneously distributed microbial populations in tested rhizospheric soils may probably be due to the differences in physico-chemical properties of soils for example, pH, temperature, moisture content, organic matter content (Burdman et al., 2001; Kennedy et al., 2004; Kennedy et al., 2005) and the nutrient pool of soils supporting the growth of microbial populations. Additionally, the diffusible metabolites released by the plant genotypes into the surrounding rhizosphere and the consequent uptake of such exudates (for example sugars, aminoacids, proteins, flavonoids etc.) as a source of carbon and energy (Skorupska et al., 2010) by microbial communities might have accounted for greater populations in rhizospheres than did the non rhizosphere regions (Zak et al., 2003; Broeckling et al., 2008).

5.2 Characterization of plant growth promoting rhizobacteria

In this study, a total of 250 bacterial cultures including nitrogen fixers (symbiotic N_2 fixers =150 and asymbiotic N_2 fixers=50) isolated from chickpea, pea, greengram and lentil nodules and PSB (N=50) recovered from various rhizospheres were characterized morphologically and biochemically (Holt et al., 1994). Later on, of the total PSB (N=50), only eight bacterial cultures isolated from mentha, chilli, cabbage

and mustard rhizospheres were subjected to 16S rRNA gene sequence analysis to identify them to species level (Table 4). The rhizobial strains (N=150) belonged to genera *Mesorhizobium* (chickpea), *Bradyrhizobium* (greengram) and *Rhizobium* specific to both pea and lentil identified by plant infection test. Wani et al., (2008c) and Ahemad and Khan (2011a) in a similar study have also reported the presence of *Mesorhizobium* sp. in chickpea, *Rhizobium* sp. in pea and *Bradyrhizobium* sp. in greengram nodules. Likewise, numerous workers across different regions have also isolated PSM including PSB (Khan et al., 2010) and PSF (Khan et al., 2009) from different rhizospheres for example *P. putida* (Ahemad and Khan 2012) and *Pseudomonas*, *Aeromomnas*, *Klebsiella* and *Enterobacter* from chickpea, mustard and wheat rhizosphere (Kundu et al., 2009). The isolated rhizobial strains were found as Gram negative while PSB showed a variable Gram reaction. In other studies numerous Gram negative/positive bacteria such as *Pseudomonas/Bacillus* (Wani et al., 2007; Ahemad and Khan 2012) and *Burkholderia* sp. have been isolated and characterized from both conventional and contaminated sites (Arora and Jain 2012). Similarly, Gram negative, aerobic, non spore forming motile and rod shaped *R. halotolerans* sp. nov. from chloroellyenes contaminated soil (Diange and Lee 2013) have been recovered. Furthermore, all the bacterial cultures in general showed a variable biochemical characteristics which is in agreement with other findings (Ahemad and khan 2009; Oves et al., 2013).

5.3.1 Functional diversity of PGPR

Plant associated bacteria able to colonize the plant roots aggressively and facilitating plant growth are in general called as PGPR (Kloepper et al., 1998). Such PGPR belonging to different functional groups including broadly the nitrogen fixers (Star et al., 2012) and phosphate solubilizers (Khan et al., 2013) have been found to exhibit a variable effect on plant growth that may range from neutral (Dudeja et al. 2012) to beneficial (Liu et al., 2012b) to deleterious (Akello et al., 2007). However, inoculation/natural benefits of such PGPR to plants could be realize only when the functionally variable strains of PGPR forms a strong association with plant roots (colonization) and survive and establish in the rhizosphere or as an endophyte (Lakshmanan and Baism 2013). Considering the functional diversity and its importance in plant growth promotion, a wide array of microbial cultures including bacteria, fungi and actinomycetes have been isolated by huge number of workers from different regions of the world. Despite the fact that the exact mechanisms by which

the PGPR augment plant growth are not fully understood, it is generally accepted that PGPR promotes the growth of plants by numerous direct or indirect mechanisms (Glick 1995). Of these, the plant growth may directly be influenced by BNF (Figueiredo et al., 2007; Remans et al., 2008b); synthesis of siderophores (Katiyar and Goel 2004; Wani et al., 2007a; Wani et al., 2008) solubilization of minerals such as phosphorous (Khan et al., 2009; Khan et al., 2010), or synthesis of plant hormones, such as auxins (Rodrigues et al., 2008, Indiragandhi et al., 2008), gibberellins (Gutie´rrez Manero et al., 2001); or plant hormone regulators, such as ACC deaminase (Ganesan 2008, Jiang et al., 2008). On the contrary, plant growth may indirectly be promoted by PGPR by exerting deleterious effects against phytopathogenic organisms (Zehnder et al., 2000; Khan et al., 2002). Here in this study, the focus was onto assessing the functional diversity among isolated PGPR and to apply them as inoculant for raising/enhancing the production of legumes in order to reduce the dependence on chemical fertilizers, if any.

Of the total 250 bacterial cultures isolated from different sources, a total of 100 bacteria involving symbiotic N_2 fixers (N=50) and asymbiotic N_2 fixers (N=20) and PSB (N=30 including eight molecularly characterized PSB) were assayed for functional variations among PGPR. The bacterial cultures belonging to different genera showed differing PGP activities and each bacterium demonstrating varied activities was assigned different groups. Nitrogen fixers and P-solubilizers in general, were divided into six PGP groups where eight PGP traits were considered for grouping symbiotic nitrogen fixers and phosphate solubilizers where as seven PGP traits were considered while grouping asymbiotic nitrogen fixers for example *Azotobacter*. The production of similar plant growth promoting substances by PGPR including N_2 fixers like *Rhizobium* (Ahmad et al., 2008; Garcia-Fraile et al., 2012), *Bradyrhizobium* (Atieno et al., 2012), *Mezorhizobium* (Zhang et al., 2012), *Ensifer* (Zhou et al. 2013), *Sinorhizobium* (Galardini et al. 2011) and free living P-solubilizers for example *Bacillus* (Zaidi et al., 2006; Wani et al., 2007c), *Pseudomonas* (Ahemad and Khan 2012), *Achromobacter* (Jha and Kumar 2009). *S. maltophilia* (Zhu et al., 2013) and other bacteria (Ghyselinck et al., 2013; Kavamura et al., 2013) and their subsequent placement in different functional groups have been reported.

5.3.2 ACC deaminase activity of PGPR

Of the functionally diverse microbes, the bacterial genera capable of producing ACC deaminase has become important due in part to their ability to play significant roles in the root morphogenesis of different plants like maize and wheat (Shahroona et al., 2006b; Shahroona et al., 2008) and nodulation of various legume plants (Glick et al., 2009; Nascimento et al., 2011b). Realizing the importance of ACC deaminase in crop production, the present study was aimed at determining ACC deaminase activity of rhizobia (isolated from nodules) and P-solubilizing bacteria (recovered from rhizospheric soils) using DF salt medium containing 3 mM ACC instead of $(NH_4)_2SO_4$). Interestingly, both rhizobia (N=24 strains) involving *Mesorhizobium* (N=11), *Rhizobium* specific to pea (N=7), *Bradyrhizobium* (N=2) and *Rhizobium* specific to lentil (N=4) and P-solubilizers (N=17) synthesized a variable but detectable amounts of ACC deaminase, as also reported by others (Penrose and Glick 2003; Zahir et al., 2011). Among rhizobia, *R. leguminosarum* (RP2) produced highest (238 µmol α ketobutyrate/mg protein/h) amounts of ACC deaminase where as among P solubilizers *P. putida* (PSE3) showed maximum (652 µmol α ketobutyrate/mg protein/h) production of ACC deaminase. While comparing the ACC deaminase produced maximally by the two bacterial strains *in vitro*, *P. putida* showed 2.7 fold increases in ACC deaminase activity compared to those observed for *R. leguminosarum* strain RP2. A similar variation in ACC deaminase activity among different rhizosphere and phyllosphere microbes (Bal et al., 2013) and even among endophytes (Karthikeyan et al., 2012) and nodule forming rhizobia are reported (Ma et al., 2003a; Glick 2005), It is generally accepted that the organisms capable of producing larger quantity of ACC deaminase binds relatively non-specifically to a range of plant surfaces and shows no preference to a specific plant genotype. In contrast, the organism expressing low level of ACC deaminase activity binds specifically to plant tissues and in turn prevent a substantive increase in ethylene level (Glick 1995). Numerous PGPR containing ACC deaminase have been shown to improve growth of plant root by inhibiting ethylene synthesis and consequently affects nodulation and growth (Glick et al., 2007).

5.3.2 Phosphate solubilization

Phosphorus (P) is one of the most essential macro-elements required for growth and development of plants. It is essentially required for various physiological functions of plants for example- (i) energy transfer (ii) signal transduction (iii) macro-molecular biosynthesis (iv) photosynthesis and (v) respiration by plants (Saber et al., 2005; Fernández et al., 2007; Khan et al., 2009). The deficiency of P in contrast severely restricts growth and yields of plants. The P deficiency problems however, are generally circumvented by applying P fertilizers by field practitioners, whom they use to achieve optimum plant productivity; but this practice is expensive. The excessive amounts of phosphatic fertilizers applied to soils is reported to alter the microbial composition and functions and hence, the soil fertility (Gyaneshwar et al., 2002). For instance, the long-term effect of different sources of phosphate fertilizers, like single superphosphate, North Carolina phosphate rock, partially acidulated North Carolina phosphate rock and diammonium phosphate on microbial activities, such as basal respiration, substrate-induced respiration, inhibition of substrate-induced respiration by streptomycin sulphate (fungal activity) and actidione (bacterial activity) and microbial biomass C of pasture soils is reported. In the field experiment, the fertilizer addition had no significant effect on basal respiration but increased substrate-induced respiration and microbial biomass C (Bolan et al., 1996). Similarly, the application of triple superphosphate (94 kg/ha) has shown a substantial reduction in microbial respiration and metabolic quotient (qCO_2) (Chandini and Dennis 2002). The community structure of both fungi and bacteria was significantly affected by phosphate suggesting that phosphate application may be an important contributor to microbial community structural change during agricultural management (Rooney and Clipson 2009). As a result, the plant suffers heavily from P deficiency in soils. Organic P on the other hand also constitutes a large fraction of soluble P, as much as 50% in soils with high organic matter content (Oberson et al., 2001; Bishop et al., 1994; Barber 1984). Emphasis is therefore, being placed onto the possibility of greater utilization of unavailable P forms wherein the P-solubilizing microbes could play a pivotal role in making soluble P available to plants. Current developments in sustainability therefore, involve a rational exploitation of soil microbial activities and the use of less expensive, though less bioavailable, sources of plant nutrients, like rock phosphates (RP), which may be made available to plants by microbiologically mediated processes (Rajankar et al., 2007; Bojinova et al., 2008; Oliveira et al., 2009).

Therefore, the use of microbial inoculants (biofertilizers) possessing P-solubilizing activities in crop productivity is considered as an environment-friendly alternative to further applications of mineral P fertilizers. The PGPR strains isolated in this study were therefore screened and assessed for P solubilizing activity using both solid and liquid Pikovskaya medium.

In this study, a total of 250 bacterial strains were screened for PSA on solid Pikovskaya medium containing TCP. Of these, a total of 69 PGPR strains belonging to different genera showed distinct PSA on solid Pikovskaya plates, as also reported by others (Liu et al. 2012; Roca et al. 2013). Of the total 69 bacterial strains exhibiting PSA on solid Pikovskaya plates, 49 bacterial strains belonging to rhizobia, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Enterobacter* and *Achromobacter* were quantitatively assessed for P solubilization in liquid Pikovskaya medium containing 5 g/l TCP. The size, solubilization index (SI) and quantity of P solubilized both on solid and in liquid medium, however, differed greatly among bacterial strains. Generally the PGPR strains tested in this study solubilized substantial amounts of TCP in liquid Pikovskaya medium with concomitant drop in pH of the culture medium. Interestingly, the eight rhizobial strains also solubilized TCP that ranged between 45 µg/ml (*Rhizobium* sp. RP6) to 148 µg/ml (*Bradyrhizobium* sp. RB6). While among non-symbiotic nitrogen fixers the amounts of TCP solubilized varied between 87 µg/ml (*Azotobacter* sp. AZ20) to 215 µg/ml (*Azotobacter* sp. AZ19) and 111 µg/ml (*Enterobacter* sp. PSE26) to 321 µg/ml (*Achromobacter* PSE28) among other P solubilizers. The solubilization of insolusble P by the rhizosphere microorganisms and concurrently decrease in pH of the medium has often been due to the secretion of organic acids (Khan et al., 2007). Similar evidence of phosphate solubilization under conventional environment by *Bacillus*, *Pseudomonas* and other microbial communities have been reported (Kumar et al., 2008; Poonguzhali et al., 2008; Rajkumar and Freitas 2008) is reported.

5.3.4.1 Bacterial biosynthesis of indole acetic acid

The plant hormones (phytohormones) for example IAA regulate a whole repertoire of plant developmental process as reported by several workers (Khalid et al., 2004; Spaepen and Vanderleyden 2011). Conceptually, the phytohormone, IAA is reported to control cell division, cell enlargement, root initiation, growth rate, phototropism, geotropism and apical dominance in plants (Khan et al., 2009; Ahemad and Khan 2011b). Apart from such physiological roles, there is also reports

suggesting that the IAA may also act as a signalling molecule and affects gene expression in some microorganisms (Spaepen and Vanderleyden 2011). In addition, IAA has also been found to play some major roles in the onset of symbiosis in legumes by acting as a signalling molecule (Barker and Tagu 2000). Effect of IAA (both bacterial and plant origin) on plants however, depends upon the amount of IAA produced and the sensitivity of the plant tissue to changes in IAA concentration. However, perhaps, less well known is the fact that some microorganisms also produce auxin (Costacurta and Vanderley 1995). In order to better understand this hypothesis of IAA production by PGPR, the experiments were designed to search the IAA producing ability of PGPR and to find some novel IAA producing bacterial strains which could later on be used as inoculant and applied to facilitate plant growth and development in different production systems. Generally, the secretion of IAA by all bacterial strains increased tremendously in this study with increasing concentrations of inducer molecule tryptophan which however, decreased at certain higher doses of tryptophan. The maximum amount of IAA produced by RB6 strain of *Bradyrhizobium* sp. among rhizobia was 92 µg/ml while minimum was 64 µg/ml when *M. ciceri* RG5 was grown in LB medium treated with 100 µg tryptophan/ml, respectively. In a follow up study, PGPR other than rhizobia tested in this study, showed a variable production of IAA under changing concentrations of tryptophan. In agreement to these findings there are also numerous reports on IAA synthesis by PGPR strains (Ahmad et al., 2008; Ahemad et al., 2010b). For example, rhizobia isolated from *Vigna mungo* nodules (Mandal et al., 2009) and *B. japonicum* collected from soybean nodules (Boiero et al., 2007) have been shown to secrete IAA. While comparing the IAA synthesizing efficiency of all rhizobabacterial strains together, the IAA was synthesized maximally by *Azotobacter* sp.

5.3.4.2 Time and tryptophan concentration dependent production of IAA

Indole acetic acid released by the representative strains of three groups of chosen rhizobacteria such as symbiotic and asymbiotic N_2-fixers, and P-solubilizers was assayed using LB broth treated with a varying concentrations (50, 100, 200, 400 and 500 µg/ml) of tryptophan at different intervals (4, 8, 12 and 16 days). The amount of IAA produced differed among PGPR isolates and were concentration and time dependent. Generally, the amount of IAA synthesized by bacterial strains increased with increasing concentration of tryptophan which however depressed at the highest

test concentration of tryptophan. As an example, *Rhizobium* strain RV9 produced IAA 65, 74, 90, 115 and 123 µg/ml at 50, 100, 200, 400 and 500 µg/ml tryptophan concentration, respectively after four days of growth at 28±2 °C. However, at higher concentration of tryptophan (500 µg/ml) there were some marginal decline in IAA for example in case of *Pseudoxanthomonas* strain ES4 which synthesize 55 µg/ml IAA at 500 µg/ml tryptophan concentration. This finding is in agreement to those reported by others (Arshad and Frankenberger 1991; Ahemad et al., 2011a) who have also observed a relatively low level of IAA by PGPR.

5.3.5 Siderophore production

Production of siderophores by plant growth promoting rhizobacteria is yet another important biological trait which may indirectly affect the performance of plants in any production systems. Siderophores, low-molecular mass iron chelators, synthesized by microbial communities of soil supply iron to plants that possess the mechanisms for its uptake under iron-deficient conditions (Indiragandhi et al., 2008). Mechanistically, siderophores bind to the available form of iron Fe^{3+} in the rhizosphere and makes it unavailable to the phytopathogens and consequently protects the plant health. By causing disease suppression, PGPR confers a competitive advantage to biocontrol agents for the limited supply of essential trace minerals in natural habitats. Additionally, siderophores may directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria and may function in local and systemic host resistance in plants (Joseph et al., 2007; Wani et al., 2008; Sinha and Mukherjee 2008). Therefore, realizing the importance of siderophores in the management of certain plant diseases, the production of siderophore was assayed both qualitatively and quantitatively using CAS agar and ethyl acetate extraction method in this study. Both the quality and quantity of siderophores produced by PGPR (N=50) however, varied significantly among strains. The variation in the intensity and size of orange halo produced by each bacterial strain could be due to the differences in the metabolic ability and genetic composition of each bacterium. As an example, Carlton et al., (2007) attempted to unravel the genetic basis of such differing capacity of siderophore production by bacteria and characterized genes required for ferrichrome utilization (fhu genes) in *Mesorhizobium* strain R88B, an $Fhu^{(+)}$ member of the population. From their study it was hypothesized that the ferrichrome transport system in *Mesorhizobium* strain R88B

evolved through cycles of gene acquisition and deletion, with the positive selection pressure of an iron-poor or siderophore-rich environment being offset by the negative pressure of the outer membrane receptor being a target for phage. Quantitatively, *Bradyrhizobium* sp. (vigna) strain RB2, *M. ciceri* strains RG7 and *R. leguminosarum* RP6 and *Rhizobium* (lentil) among rhizobia showed maximum but equal production of DHBA (16 µg/ml) where as SA was maximally synthesized by strain RB2 of *Bradyrhizobium* sp. (vigna). Among non-rhizobial strains, *Azotobacter* in general produced higher amounts of DHBA while SA was produced maximally by PSB. Similar evidence of siderophore production by *Azotobacter*, *Bacillus* and *Pseudomonas* (Dey et al., 2004; Poonguzhali et al., 2008, Rajkumar and Freitas, 2008) and *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* (Khandelwal et al., 2002; Wani et al., 2007b) is also reported. The ability of rhizobial strains involving both symbiotic (rhizobia) and asymbiotic nitrogen fixers (for example *Azotobacter*) and other phosphate solubilizers to produce siderophore as observed in this study suggests that such strain could also be used as a biological control agent for optimum crop production, if applied properly under different agro-ecosystems.

5.3.6 Exo-polysaccharide production

The production of EPS by PGPR is another vital characteristics which is reported to play important roles in protecting cells from (i) desiccation (ii) phagocytosis and (iii) phage attack besides their critical functions in N_2 fixation such as legume root infection and nodulation (Chen et al., 1985: Leigh et al., 1988; Spaink 2000) by preventing high oxygen tension (Tank and Saraf 2003). Furthermore, the bacteria capable of producing higher amounts of EPS have also shown a stronger ability of phosphate solubilization compared to non-EPS producing strains (Yi et al., 2008). Considering the importance of EPS, the present study was aimed at identify high EPS producing PGPR strains while growing nodule bacteria and phosphate solubilizers in basal medium supplemented with 5% sucrose. Generally, all the test PGPR strains produced a variable amount of EPS, which however differed from strain to strain.

5.3.7 Ammonia and hydrogen cyanide production

The secondary metabolites such as cyanide and ammonia secreted by majority of PGPR strains using glycine and cyanogenic glycosides (Mehnaz et al., 2011; Lim et al. 2012) have been reported in root exudates of plant (Curl and Truelove 1985). Of these secondary metabolites, cyanide in particular is produced by large number of

microorganisms across different genera and plays some important role in biological control of pathogens (Bano et al., 2003; Guo et al., 2004). On the other hand, the ammonia released by the bacterial strains play a signaling role in the interaction between PGPR and plants (Becker et al., 2002). Moreover, the ammonia released by the bacterial strains is known to increase the glutamine synthetase activity (Chitra et al., 2002). In addition, the ammonia transporters found in several PGPR are thought to be involved in the re-absorption of NH_4^+ released as a consequence of NH_3 diffusion through the bacterial membrane (Van Dommelen et al., 1997). The PGPR strains were therefore tested in this study to evaluate the synthesis of ammonia and HCN using peptone water and HCN induction medium, respectively. Of the total PGPR (N=100) involving both N_2 fixers and P-solubilizers, 51% bacterial isolates showed a positive reaction to HCN while all strains were positive to ammonia. However, the degree of HCN and NH_3 synthesized varied greatly among PGPR strains isolated from different rhizospheres. The NH_3 and HCN producing bacteria have also been reported in other studies (Devi et al., 2007; Wani et al., 2007a; Flythe and Kagan 2010; Kagan and Flythe 2012).

5.3.8 Antifungal activity

In this study, all PGPR (N=100) were tested for their antifungal activity in Petridish bioassay (Table 33, 34). Of these, the supernatant prepared from a total of 38 bacterial cultures involving symbiotic (N=10), and asymbiotic nitrogen fixers (N-11) and other PSB (N=17) showed antagonistic potential against three phytopathogens namely *Rhizoctonia* sp., *Penicillium* sp. and *Rhizoctonia* sp. in agar well diffusion method. However, antagonistic activity widely differed among bacterial strains and was found to be pathogen specific. For example, *P. putida* showed the largest zone of growth inhibition against *Rhizoctonia* sp. (19 mm) which was 27 and 47% increase over zone sizes of *Alternaria* sp. (15 mm) and *Penicillium* sp. (13 mm), respectively (Table 34). In the present study, *Rhizoctonia* sp. in particular was found as most sensitive strain (mean inhibition zone size 25.2 mm) compared to other test fungi. In contrast, the growth of *Penicillium* sp. among all three tested fungi was poorly inhibited by majority of the test PGPR strains. Similarly, rhizobia, strains of *Azotobacter* and other P solubilizers profoundly inhibited the growth of the test fungi. The variation in the antagonistic activities among different PGPR could be due to the differences in their ability to produce varying level and variable composition of secondary metabolites

(Okubara and Bonsall 2007). The antagonistic activity of rhizobia (Avis et al., 2008), *Azotobacter* (Ahmad et al., 2008) and P-solubilizer (Vassilev 2006) could also be due to the expression of low molecular weight substances for example, siderophores (Hofte and Altier 2010), molecular peptides (Ongena and Jacques 2007), enzymatic activity (cellulase etc.) (Avis et al., 2008), ACC deaminase activity (Glick 2012), and production of cyanogenic compounds (Okubara and Bonsall 2007). A similar antagonism of PGPR against phytopathogenic fungi is reported (Khan and Zaidi 2002).

5.4 Antibiotic sensitivity of PGPR

Low level resistance to a range of antibiotics was used to characterize the isolated and cultured bacterial cells in the present investigation. The results on the antibiotics sensitivity/resistance pattern (Table 34) of the cultured organisms confirmed the validity of this technique. However, the minimum inhibitory concentrations of the antibiotics varied greatly among isolated bacterial cultures. The variation in the antibiotic sensitivity/resistance among PGPR could be due to several reasons. Some argue that it is mainly due to the differences in the genetic makeup and biochemical composition of the organisms while others believe that this could be sue to loss or gain of antibiotic resistance markers among bacterial population and many a times this has been expanded by the workers around the world (Maksomov et al., 2011; Vivek eta al., 2013).

5.5 Molecular characterization

In this study, a total of eight plant growth promoting rhizobacteria expressing higher ACC deaminase activity and phosphate solubilizing potentials were subjected to 16S rRNA gene sequence analysis and using BLAST programme they were identified to species level. For this, the molecular characterization was done commercially by Macrogen Inc., Seoul, South Korea. The 16S rRNA partial genome sequences were then submitted to NCBI nucleotide based GenBank and accession number was obtained for each sequence. The bacterial strains were identified as *Pseudomonas putida* strain PSE3 (Gene Bank accession number HM236047) and PSE5 (Gene Bank accession number HM236047), *Achromobacter* strain ES1 (Gene Bank accession number *JX483710)* and *ES6* (Gene Bank accession number *JX 965905*), *Enterobacter* strain ES2 (Gene Bank accession number JX 965901),) *Bacillus pumilus* strain ES3 (Gene Bank accession number JX 965902), *Pseudoxanthomonas* strain

ES4 (Gene Bank accession number JX 965903) and *Stenotrophomonas* strain ES5 (Gene Bank accession number JX 965904), respectively (Table 36).

5.6.1 Biological properties of legumes

Favourable effects of inoculation with plant growth promoting rhizobacteria especially the nitrogen fixing and the phosphate solubilizing microorganisms and incredible enhancement in the production of crops for example legumes have widely been reported by many workers (Zaidi et al., 2003; Wani et al., 2007c; Basak and Biswas 2010; Yu et al., 2012; Shahzad et al., 2013). Of the heterogeneously distributed microbial communities within soil ecosystems, the phosphate solubilising microorganisms converts the unavailable forms of P (organic and inorganic P) into soluble P for uptake by plants (Khan et al., 2009; Khan et al., 2010). In addition, the phosphate solubilizing microbes produce good quantity of different plant growth promoting substances for example IAA (Marques et al., 2010), siderophores (Mitter et al., 2013), ACC deaminase (Zahir et al., 2011) and ammonia and cyanogenic compounds (Bano et al., 2003) which may improve plant health and stimulate the microbial activity in rhizospheric soils of different crops. The combined inoculations of nitrogen fixing [symbiotic for instance rhizobia and asymbiotic for example *Azotobacter*] and P-solubilizers like *Bacillus* (El-Nagdy et al., 2010) *Pseudomonads* (Jha et al., 2011), *Enterobacter* (Deepa et al., 2011) *Burkholderia* (Mitter et al., 2013) etc. have been found superior over many isolated application of single culture and have shown dramatic increase in crop yields in different production systems (Mishra et al., 2011; Yu et al., 2011). The use of single or composite culture of PGPR and rhizobia in maintaining the soil nutrient pool and hence, enhancing the crop production has become important in sustainable agricultural practices. Considering the importance of natural and inexpensive soil microflora in enhancing the overall performance of crops, the present study was designed with specific objective to find microbial pairing/sole culture which can later on be used as inoculant for protein rich crops, the legumes. Due to the conflicting reports on the effects of PGPR on plants across different regions, the present investigation was undertaken to assess the impact of isolated PGPR here in this study onto the four popularly grown legumes like chickpea, pea and greengram and lentil. The expression of multiple growth mediating substances by PGPR strains as observed under *in vitro* experiments formed the basis of the selection of such potential PGPR strains for test against legumes

grown under both pot and field soils treated with/without recommended rates of urea and diammonium phosphate (DAP).

The application of recommended rates of synthetic fertilizers such as urea and DAP in general, did not have any significant ($P \leq 0.05$) effect on the biological (Table 86) and chemical properties (Table 87 and 88) of chickpea, pea, greengram and lentil grown in alluvial soils compared to those of single or composite application of microbial cultures. However, while comparing the effects of the two fertilizers, DAP in particular showed a profound impact on the measured parameters of the test legumes. For example, DAP when applied alone marginally increased the whole biomass of chickpea by 2 (pot) and 3% (field), of pea by 9 (pot) and 7% (field), of greengram by 5 (pot) and 5% (field) and lentil biomass by 2 (pot) and 5% (field) at harvest (Table 86) over urea application. Similarly, the sole application of microbial cultures like *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* specific to each legume showed superior impact than the single application of either urea or DAP on the measured parameters of chickpea, pea, greengram and lentil plants. As an example, *P. putida* had an obvious stimulatory effects on dry matter accumulation in all legumes and hence, enhanced the total dry biomass by 8 (pot) and 7% (field), pea dry matter yield by 12 (pot) and 8% (field), greengram biomass by 15 (pot) and 17% (field) and lentil biomass by 8 (pot) and 13% (field) at harvest (Table 86) over DAP. Furthermore, the microbial cultures were applied together with either urea or DAP while growing legumes in pot and field experiments. In this context, DAP was always applied with nitrogen fixers considering the intrinsic ability of nitrogen fixers to supply N to legumes while DAP could provide the P to growing legumes. In contrast, urea was used with other P solubilizers realizing the fact that urea will provide soluble form of N while the PSB though solubilization process are likely to provide P to the legumes. Based on this hypothesis, when the impact of microbial cultures applied together with urea/DAP was compared with those of the independent application of either urea or DAP and sole application of bacterial cultures, it was found that the measured parameters did not differ significantly ($P \leq 0.05$) among treatments (Table 86). Furthermore, the biological characteristics such as root length, shoot length, root dry biomass and shoot dry biomass was positively correlated as shown in Table 90 and Fig. 60, 61, 62 , 63, 64 (chickpea), 65, 66, 67, 68 (pea) 69, 70, 71, 72 (greengram), 73, 74 and 75 (lentil).

The co-inoculation effects of the selected bacteria namely, *P. putida, Bacillus, Azotobacter and M. ciceri* (chickpea), and *P. putida, B. pumilus, Azotobacter* and *R. leguminosarum* (pea) *P. putida, B. pumilus, Azotobacter* and *Bradyrhizobium* sp. (vigna) and *P. putida, B. pumilus, Azotobacter* and *Rhizobium* sp. (lentil) on biological and chemical characteristics of pea plants grown in sandy clay loam soils was assayed further. The composite application of the PGPR strains showed more significant increase in the biological properties such as the length of roots and shoots and concomitantly enhanced the dry matter accumulation in chickpea, pea, greengram and lentil plants. The observed benefits following dual inoculations on legumes may be due to the cumulative effect of these organisms which provided N (nitrogen fixers) and available P (PSB) and improve nutrient absorption in addition to growth promoting substances. Such increase in plant characteristics could also be due to the secretion of IAA and ACC deaminase by the applied bacterial cultures in this experiment. The phytohormone, IAA is reported to control cell division, root initiation, phototropism and apical dominance in plants (Khan et al., 2009) and PGPR for example *Pseudomonas*, *Serratia* and *R leguminosarum* (Zahir et al., 2011) containing ACC deaminase induce metabolic changes (Stearns et al., 2012) and hence, increase the growth of plants by inhibiting/reducing ethylene synthesis and also improves the nodulation and growth of legumes (Glick et al., 2007; Glick 2014). Ethylene, one of the important plant hormones is reported to be involved in the regulation of many physiological processes (Arshad and Frankenberger 2002) and also affects nodule forming ability of legumes and even acts as a stress hormone. However, ethylene at higher concentrations restricts plant growth (Grichko and Glick 2001). The inhibitory effect of ethylene is however, abated by the ACC deaminase (Madhaiyan et al., 2006) by hydrolyzing ACC to NH_3 and a-ketobutyrate (Glick et al., 1998; Safronova et al., 2006). The NH_3 so evolved is used as a source of N by bacteria and therefore, the build up of ethylene within the plant is limited. Once the level of ethylene is reduced, plant becomes stress free and grows optimally. In agreement to this finding, plants inoculated with PGPR endowed with ACC deaminase ability have also shown dramatic increase in plant growth (Shahzad et al., 2010; Murset et al., 2012). Besides these, the PGPR strains in general could synthesize catechol type siderophores, EPS and ammonia. The synthesis of EPS by the bacterial strains as observed in this study might have promoted root colonization and consequently root ramification and nodulation (Hirsch 1999). However, the combination of *Azotobacter*

with other bacterial cultures in some cases was found inferior relative to the combination of microbes other than *Azotobacter* or the combination of two synthetic fertilizers which could possibly be due in part to its inability to supply N to legumes or its ability not to synthesize ACC deaminase. Similar negative impact of *Azotobacter* on legumes has also been reported by Streeter and Wong (1988). The result indicates that the combination of *Rhizobium* sp. and *Bacillus* in particular was highly effective than other single or dual inoculation or mixture of fertilizer treatments which could be used for improving the yield of legume crops.

5.6.2 Chlorophyll content

Chlorophyll is the most important photosynthetic pigment which plays an important role in converting light energy into chemical energy. Chlorophyll molecule has a cyclic tetrapyrrolic structure (porphyrin) with an isocyclic ring containing a magnesium atom at its centre and a phytol chain attached to it. Considering the importance of cholorophyll in overall development of plants, the impact of single or simultaneous inoculation of N_2-fixer and P-solubilizers or recommended rates of nitrogenous (urea) and phosphatic fertilizers (DAP) on the chlorophyll content of fresh foliage of chickpea, pea, greengram and lentil was determined at flowering stage of each legume. Generally, P-solubilizers (*P. putida*, *B. pumilus* and *Azotobacter*) when used in combination with N-fixers (rhizobia) had the most identifiable effects and profoundly increased the chlorophyll contents of each legumes relative to other inoculated/uninoculated plants grown in soils treated with/without synthetic fertilizers. For example, in pea plant co-inoculated with [*B. pumilus* with *R. leguminosarum*] the chlorophyll content was increased by 51 % in pot trial while it 129% in field soils over uninoculated and untreated control. Likewise, the composite application of [*B. pumilus* and *R. leguminosarum*] increased the chlorophyll content by 5% in pot grown pea plants while it was 9% increase when the inoculated pea was grown in field soils, over plants grown in soils treated only with 20 kg/ha urea with 90 kg/ha DAP under both pot and field trials (Table). Similarly in other experiments the co- inoculation of P-solubilizer and N-fixer has shown tremendous improvement in the photosynthetic content in other legumes (Wani et al., 2007c). The biological characteristics like chlorophyll content was strongly and positively correlated in pot and field grown chickpea as shown in Table 90 and in Fig. 77 (chickpea), 78 (pea), 79 (greengram) and 80 (lentil).

5.6.3 Symbiotic characteristics

Since the discovery of plant symbiotic haemoglobins (Hbs) in soybean root nodules, Hbs have been identified in a range of plants and are now believed to exist in all plants. The symbiotic haemoglobins commonly known as Lb exist abundantly in the root nodules of legumes. It binds O_2 with high affinity and is thought to fulfil two functions- (i) limits O_2 concentration in the root nodule to a level at which the O_2-sensitive nitrogenase can function, and (ii) deliver O_2 to the respiring bacteroids to meet the high ATP demands of N_2 fixation (Delgado et al., 1998). Realizing the potential of Lb in an effective and functional N_2 fixation process mediated by rhizobial the present study was designed to detect Lb in fresh nodules of chickpea, pea, greengram and lentil plants grown in conventional but fertilizer amended soils. The leghaemoglobin content measured in fresh nodules of inoculated and uninoculated chickpea, pea, and greengram and lentil plants assayed at pod fill stage varied considerably among treatments (Table 86). The symbiotic characteristics (nodulation and leghaemoglobin content) of inoculated/uninoculated chickpea, pea, greengram and lentil plants grown in pot or field soils treated with or without urea/DAP was variable. Among fertilizer treatments, DAP in general showed increasing effect on nodulation and leghaemoglobin content in fresh nodules over urea but it was statistically non significant ($P \leq 0.05$). However, the sole application of rhizobia specific to each legume was found superior over other single microbial cultures or single application of urea/DAP applied to both pot and field grown legumes. For example, the sole application of rhizobia significantly enhanced the nodulation in chickpea grown in pot (58%) and field (69%), in pea 14 (pot) and 35% (field), greengram 49 (pot) and 57% (field) and lentil 27 (pot) and 38% (field) over single application of DAP. Similarly, the leghaemoglobin content in fresh nodules of each rhizobia inoculated legume grown both in pots and field were higher than those recorded for fertilizer treated soil. The co-culture of *B. pumilus* with rhizobia in particular performed exceptionally well and enhanced the nodulation profoundly compared to those determined for other single or combined microbial treatments or single or dual fertilizer application. For example, *B. pumilus* in association with *M. ciceri* significantly increased the nodule numbers in chickpea by 52% in pot and 63% in field over 30 kg urea/ha+80 kg DAP/ha. Such enhancement in nodulation following microbial inoculation could possibly be due to the synergistic impact of both nitrogen fixers and P solubilizers. During this interactive association, rhizobia in

particular might have supplied the required amount of N to the chickpea plants while PSB for example *Bacillus* in this case might have provided soluble P to legumes. Interestingly, even the rhizobial strain used in this study was able to solubilise insoluble P and showed maximum increase in nodulation under both pot and field environments. These two key element together thus resulted in enhanced symbiotic attributes of chickpea plants. However, apart from N and P (Richardson et al. 2009; Khan et al., 2013) supplied by N fixer and PSB respectively, there are also possibility that the secretion of other growth promoting substance by these cultures under both pot and field environment might have contributed the enhanced symbiosis and hence the symbiotic characteristics of legumes in general. Furthermore, IAA by acting as phytohormone improves various stages of legumes (Khan et al., 2009) including the symbiotic process (Boiero et al., 2007; Remans et al., 2008; Ali et al., 2009). Similar increase in symbiotic attributes of legumes following microbial inoculation has been reported by many workers (Mishra et al. 2011; Yu et al., 2012). Nodule number and leghaemoglobin content were strongly and positively correlated for pot and field grown as shown in Table 90 and Fig. 80 and 84 (chickpea), 81 and 85 (pea), 82 and 86 (greengram) and 83 and 87 (lentil).

5.6.4 Nutrient uptake

Inoculation of legumes with PSB and nitrogen fixers (both rhizobia and *Azotobacter*) considerably increased the N and P accumulation within roots and shoots of chickpea, pea, greengram and lentil plants grown in soils treated with/without chemical fertilizers. The increase in P in roots and shoots of each legume could be attributed to the ability of PSBs to solubilize inorganic P of soils. Combining an improved plant nutrient supply with N (*Azotobacter*) and P (PSB) with plant growth promotion appears to have additive and possibly even multiplicative effects (factor interaction not calculated in this study). However, the variations in the effectiveness of microbial combinations in this study are probably due to the differences in the functionality of the tested microbial strains, variations in their survivability and colonization efficiency of the inoculated cultures in the soil, or strong competition from the natural microbiota of field soils, leading possibly to the exclusion of the inoculated cultures from the rhizospheres. Moreover, the combined inoculation effects were greater than the sum of the individual inoculation effects, suggesting synergism beyond simple additive effects (positive multiplicative interaction). For example, the highest increase in concentrations of N and P was recorded with *B. pumilus* with *Bradyrhizobium* in

root and shoots of greengram plants over DAP application (Table 87 and 88). In contrast, the application of *Azotobacter* with rhizobia in general had a poor impact on N and P contents of both roots and shoots of all legumes (Table 87 and 88). These results strongly suggested that a relationship existed between root colonization, P uptake, and growth promotion.

Table 86- Comparative assessment of inoculation response on to the biological characteristics of legumes grown in alluvial soil

Treatments	Total dry biomass								Nodule No.							
	Chickpea		Pea		Greengram		Lentil		Chickpea		Pea		Greengram		Lentil	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
[a]DAP vs [b]U	2.2	2.6	8.8	7.1	5.0	5.0	2.2	5.3	6.4	3.2	0	9.0	2.9	6.1	4.8	4
P. putida vs [b]U	5.3	4.4	10.6	5.8	12.5	13.7	4.9	11.4	45.1	32.2	3.5	31.8	35.3	39.3	19.0	28
P. putida vs [a]DAP	7.8	6.8	12.1	7.5	15.2	16.5	7.6	13.3	54.8	38.7	7.1	38.6	41.1	45.4	28.6	40
B. pumilus vs [b]U	4.1	3.1	8.1	3.1	9.7	10.5	2.2	10.2	38.7	25.8	10.7	36.3	29.4	33.3	9.5	20
B. pumilus vs [a]DAP	5.3	4.4	4.4	11.1	12.5	11.4	7.1	13.6	67.7	74.1	14.2	47.7	52.9	66.6	33.3	44
Azotobacter vs [b]U	3.0	1.7	1.7	-1.2	7.0	8.3	2.6	5.7	36.3	28.1	3.6	20.8	31.4	31.4	13.6	23.0
Azotobacter vs [a]DAP	5.4	4.1	3.0	0.4	9.6	10.9	5.2	7.5	45.4	34.3	7.1	27.0	37.1	37.1	22.7	34.6
[c]Rh vs [b]U	1.9	0.5	-0.7	-3.7	4.4	5.2	0	4.6	30.3	21.8	10.7	25.0	25.7	25.	4.5	15.3
[c]Rh vs [a]DAP	3.0	1.7	-4.1	3.7	7.0	6.1	4.8	7.9	57.5	68.8	14.2	35.4	48.5	57.1	27.2	38.4
P. putida + [b]U vs U+ [a]DAP	-1.7	-6.7	0.3	-14.6	-5.0	-5.0	-5.1	-2.8	15	31.4	10.1	28.8	20.5	27.0	13.	32.1
B. pumilus + [b]U vs U+ [a]DAP	-1.5	-4.5	1.7	-13.3	-2.8	-2.1	-3.	-1.8	12.5	11.4	11.8	32.6	25.6	35.1	21.	39.2
Azotobacter + [b]U vs U+ [a]DAP	-3.4	-7.8	-1.7	-16.8	-7.9	-7.9	-8.0	-4.6	10	25.7	3.3	23.0	7.7	18.9	4.3	25
[c]Rh +DAP vs [b]U + [a]DAP	-0.9	-0.4	-0.8	-3.5	-0.3	-0.3	-0.3	0.3	12.5	45.7	23.7	34.6	43.5	51.3	26.0	39.2
P. putida + [c]Rh vs [b]U + [a]DAP	0.7	2.8	-0.3	-6.6	4.3	5.7	5.1	5.6	47.5	57.1	32.2	40.3	64.1	81.0	34.7	67.8
B. pumilus + [c]Rh vs [b]U + [a]DAP	3.5	5.7	1.1	-6.6	7.1	8.6	7.7	7.5	52.5	62.9	33.5	46.1	71.8	86.4	43.4	71.4
Azotobacter + [c]Rh vs [b]U + [a]DAP	-0.2	1.8	-1.9	-8.9	1.43	2.8	1.1	4.0	6.5	51.4	25.4	34.6	56.4	75.6	26.0	57.1

In this table values indicate percent increase or decrease (-) over each other; [a]DAP represents the recommended dose of phosphatic fertilizer applied for each legume; [b]U- indicates the recommended rate of urea applied for each legume; [a]R: indicates *Rhizobium* sp. specific to each legume

Table 87- Comparative assessment of inoculation response on to the chemical characteristics of legumes grown in alluvial soil

Treatments	Root N								Shoot N							
	Chickpea		Pea		Greengram		Lentil		Chickpea		Pea		Greengram		Lentil	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
[a]DAP vs [b]U	4.1	7.4	6.4	3.0	5.1	-2.5	8.0	12.0	6.8	18.7	2.5	-4.6	3.5	3.5	3.6	5.3
P. putida vs [b]U	16.6	18.5	12.9	6.0	12.0	12.5	16.0	16.0	17.2	28.1	5.1	6.9	8.7	10.0	10.9	8.9
P. putida vs [a]DAP	12.5	11.1	16.1	9.0	17.9	15.0	16.0	20.0	10.3	31.2	7.6	9.3	12.0	14.2	14.5	12.5
B. pumilus vs [b]U	8.33	14.8	6.4	6.0	5.0	7.5	8.0	8.0	10.3	25.0	2.5	2.3	3.5	7.1	7.2	7.1
B. pumilus vs [a]DAP	20.8	40.7	12.9	12.1	17.9	17.5	24.0	28.0	27.5	12.5	5.1	11.6	14.	19.6	18.	19.6
Azotobacter vs [b]U	12	10.3	6.	2.9	7.3	15.3	7.4	3.5	9.6	7.8	2.5	12.1	5.0	6.8	7.0	3.3
Azotobacter vs [a]DAP	8	3.4	9.0	5.8	12.1	17.9	7.4	7.1	3.2	10.5	5.0	14.6	8.4	10.3	10.5	6.7
[c]Rh vs [b]U	4	6.8	0	2.9	0.0	10.2	0	-3.5	3.2	5.2	0.0	7.3	0.0	3.4	3.5	1.6
[c]Rh vs [a]DAP	16	31.0	6.0	8.8	12.1	20.5	14.8	14.2	19.3	-5.2	2.5	17.0	10.0	15.5	14.0	13.5
P. putida + [b]U vs U+ [a]DAP	-17.0	-2.3	-2.6	-5	1.9	-3.7	-13.5	-10.8	0.0	-19.6	-2.1	-3.7	-4.0	-2.8	-2.8	0.0
B. pumilus + [b]U vs U+ [a]DAP	-4.8	-7.1	-2.6	-2.5	1.9	-1.8	-10.8	-8.1	2.2	-15.6	0.0	-1.8	-1.4	-1.4	-2.8	0.0
Azotobacter + [b]U vs U+ [a]DAP	-19.5	-4.7	-7.8	-5	-5.7	-7.4	-18.9	-13.5	-8.8	-23.5	-6.3	-7.5	-7.2	-5.7	-7.0	-2.8
[c]Rh +DAP vs [b]U + [a]DAP	-7.3	-7.1	-7.8	-2.5	-3.8	-1.8	-5.4	-5.4	-2.2	-7.8	-8.5	-3.7	-2.8	-1.4	0.0	1.40
P. putida + [c]Rh vs [b]U + [a]DAP	2.4	7.1	-2.6	2.5	5.7	5.5	2.7	5.4	6.6	3.9	10.0	11.3	2.8	4.3	2.8	4.2
B. pumilus + [c]Rh vs [b]U + [a]DAP	4.8	0	2.6	5	9.6	7.4	5.4	5.4	13.3	7.8	12.7	13.2	7.2	7.2	4.2	7.04
Azotobacter + [c]Rh vs [b]U + [a]DAP	-2.4	2.3	-2.6	2.5	-1.9	0	-2.7	0.0	2.2	-1.9	4.2	5.6	0.0	1.4	-1.4	1.40

In this table values indicate percent increase or decrease (-) over each other; [a]DAP represents the recommended dose of phosphatic fertilizer applied for each legume; [b]U- indicates the recommended rate of urea applied for each legume; [a]R; indicates *Rhizobium* sp. specific to each legume

Table 88- Comparative assessment of inoculation response on to the chemical characteristics of legumes grown in alluvial soil

Treatments	Root P								Shoot P							
	Chickpea		Pea		Greengram		Lentil		Chickpea		Pea		Greengram		Lentil	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
[a]DAP vs [b]U	14.2	26.0	8.6	0	18.	22.2	14.2	12.9	6.8	18.7	6.4	3.2	15.3	24.3	7.8	9.5
P. putida vs [b]U	28.5	47.8	13.0	4.5	33.3	37.0	28.5	22.5	17.2	28.12	9.6	6.4	17.9	29.	18.4	16.6
P. putida vs [a]DAP	33.3	52.1	17.3	18.1	40.7	44.4	39.2	32.2	10.3	31.2	12.9	0	25.6	37.8	23.6	23.8
B. pumilus vs [b]U	19.0	39.1	13.0	9.0	25.9	29.6	17.8	9.6	10.3	25	6.4	9.6	7.6	18.9	7.8	11.9
B. pumilus vs [a]DAP	23.8	26.0	17.3	9.0	25.9	25.9	28.5	32.2	27.5	12.5	12.9	9.6	15.3	21.6	23.6	21.4
Azotobacter vs [b]U	12.5	17.2	4	4.5	12.5	12.1	12.5	8.5	9.6	7.8	3.0	3.1	2.2	4.3	9.7	6.5
Azotobacter vs [a]DAP	16.6	20.6	8	18.1	18.75	18.1	21.8	17.1	3.2	10.5	6.0	-3.1	8.8	10.	14.6	13.0
[c]Rh vs [b]U	4.1	10.34	4	9.0	6.25	6.0	3.12	-2.8	3.2	5.2	0	6.2	6.6	-4.3	0	2.1
[c]Rh vs [a]DAP	8.3	0	8	9.0	6.25	3.0	12.5	17.1	19.3	-5.2	6.0	6.2	0	-2.1	14.6	10.8
P. putida + [b]U vs U+ [a]DAP	-16.2	-30.4	-8.5	-25	-6.8	-8.6	-9.09	-10.6	0	-19.6	-2.6	-5.5	0	-3.7	-7.5	-5.3
B. pumilus + [b]U vs U+ [a]DAP	-16.2	-26.0	-5.7	-19.	-2.2	2.1	-4.5	-8.5	2.2	-15.68	2.6	-5.5	5.8	3.7	-3.7	-1.78
Azotobacter + [b]U vs U+ [a]DAP	-21.6	-34.7	-14.2	-30.5	-11.3	-13.0	-13.6	-14.8	-8.8	-23.5	-5.2	-5.5	-3.9	-7.4	-15.0	-8.9
[c]Rh +DAP vs [b]U + [a]DAP	-8.10	-10.8	2.8	-19.4	-4.5	-8.6	-4.5	-10.6	-2.2	-7.84	-2.6	-5.5	-3.9	-1.8	-3.7	1.7
P. putida + [c]Rh vs [b]U + [a]DAP	5.4	4.34	-2.8	-11.1	4.5	2.1	2.2	2.1	6.6	3.9	7.8	2.7	3.9	1.8	0	1.7
B. pumilus + [c]Rh vs [b]U + [a]DAP	5.4	8.69	2.8	2.7	11.3	6.5	6.8	8.5	13.3	7.8	13.1	8.3	11.7	7.4	3.7	3.5
Azotobacter + [c]Rh vs [b]U + [a]DAP	0	0	2.8	-13.8	-4.5	-4.3	-4.5	-4.2	2.22	-1.9	2.6	5.5	0	-3.70	-5.6	-1.7

In this table values indicate percent increase or decrease (-) over each other; [a]DAP represents the recommended dose of phosphatic fertilizer applied for each legume; [b]U- indicates the recommended rate of urea applied for each legume; [a]R; indicates *Rhizobium* sp. specific to each legume

Table 89- Comparative assessment of inoculation response on to the biological characteristics of legumes grown in alluvial soil

Treatments	Chlorophyll conrent								Seed yield							
	Chickpea		Pea		Greengram		Lentil		Chickpea		Pea		Greengram		Lentil	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
[a]DAP vs [b]U	-1.1	1.1	3.5	11	-3.2	1.0	7.4	9.7	7.8	10.5	5	4.16	2.2	4.2	5.1	18.4
P. putida vs [b]U	0	1.7	0.8	14	2.1	7.1	9.5	13.0	15.7	21.0	11.0	8.3	20	23.4	7.6	28.9
P. putida vs [a]DAP	0.57	1.1	2.6	17	4.3	9.1	11.7	14.1	21.0	26.3	10	12.5	24.4	29.7	10.2	34.2
B. pumilus vs [b]U	-1.15	-1.1	-0.8	12	-1.0	6.1	6.3	10.8	10.0	15.7	6.6	9.7	11	19.1	2.5	18.4
B. pumilus vs [a]DAP	-2.3	3.4	10.7	19	5.4	9.1	11.7	16.3	13.1	23.6	8.3	13.8	13	25.5	35.8	39.4
Azotobacter vs [b]U	1.1	0.5	-2.5	2.7	5.6	6.0	1.9	2.9	7.3	9.5	6.3	4	17.	18.3	2.4	8.8
Azotobacter vs [a]DAP	1.	0	-0.8	5.4	7.8	8.0	3.9	3.9	12.1	14.2	4.7	8	21	24.4	4.8	13.3
[c]Rh vs [b]U	0	-2.2	-4.3	0.9	2.2	5.0	-0.9	0.9	2.4	4.7	1.5	5.3	8.6	14.2	-2.4	0.0
[c]Rh vs [a]DAP	-1.1	2.2	6.8	7.2	8.9	8.0	3.9	5.9	4.8	11.9	3.1	9.3	10	20.4	29.2	17.7
P. putida + [b]U vs U+[a]DAP	-1.6	-1.5	-9.5	-22.2	-3.6	-3.3	-4.2	-6.4	0	-3.6	-4.	0	-9	-4.2	-11.2	-9.2
B. pumilus + [b]U vs U+[a]DAP	-2.2	-0.5	-7.3	-20.5	-1.8	-1.6	-2.5	-4.8	-1.96	1.8	-2.3	3.2	-4.	1.4	-8.1	-4.61
Azotobacter + [b]U vs U+[a]DAP	-2.7	-3.6	-11.7	-23.3	-6.3	-6.6	-7.6	-8	-3.9	-5.4	-9.5	-2.1	-15.	-7.0	-14.5	-13.8
[c]Rh +DAP vs [b]U + [a]DAP	-2.2	-0.5	0.7	-5.5	-1.8	-1.6	-2.5	-0.8	3.9	0	-10.7	-5.4	-6.1	-2.8	-6.4	-10.7
P. putida + [c]Rh vs [b]U + [a]DAP	0	2.1	2.2	5.5	0.9	0.8	3.4	2.4	15.6	12.7	11.9	10.8	4.6	2.8	3.2	4.6
B. pumilus + [c]Rh vs [b]U + [a]DAP	0.5	2.6	5.1	9.4	2.7	2.4	5.1	4.8	21.5	16.3	13.0	14.1	12. 2	5.6	8.0	6.1
Azotobacter + [c]Rh vs [b]U + [a]DAP	-1.1	0.5	-0.73	3.8	-0.9	-0.8	1.7	0	7.8	9.0	8.3	6.5	1.5	-1.4	0	0.0

In this table values indicate percent increase or decrease (-) over each other; [a]DAP represents the recommended dose of phosphatic fertilizer applied for each legume; [b]U- indicates the recommended rate of urea applied for each legume; [a]R; indicates *Rhizobium* sp. specific to each legume

Table 90- Correlation Coefficient values (R^2) of various physiological characteristics of legumes grown in pot and field experiment

Legumes	R^2 values (pot vs field)							
	Root length	Shoot length	Root dry biomass	Shoot dry biomass	Chlorophyll	Nodule no.	Lb content	Seed yield
Chickpea	0.90	0.93	0.97	0.84	0.91	0.88	0.89	0.98
Pea	0.71	0.58	0.66	0.80	0.88	0.80	0.59	0.97
Greengram	0.98	0.95	0.98	0.99	0.98	0.99	0.98	0.97
Lentil	0.92	0.97	0.97	0.91	0.98	0.94	0.98	0.96

The correlation values of physiological characteristics of pot and field grown legumes is given in table

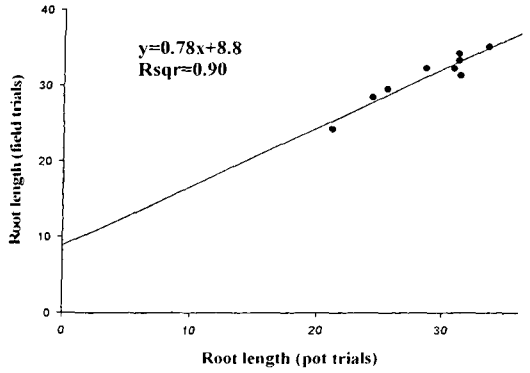

Fig. 60 Linear regression of root length of chickpea grown in pot and field trials

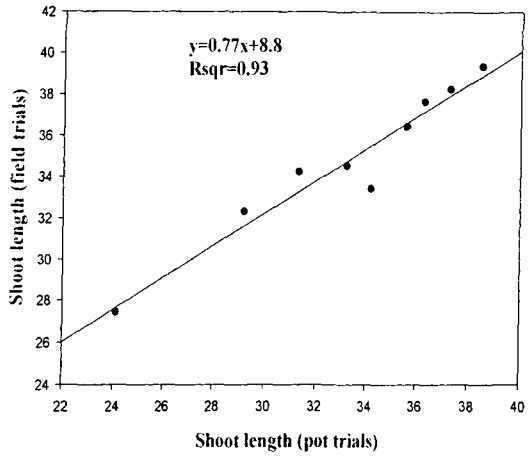

Fig. 61 - Linear regression of shoot length of chickpea grown in pot and field trials

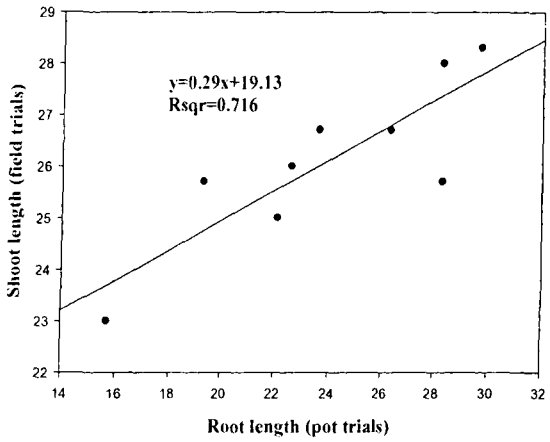

Fig. 62 - Linear regression of root length of pea grown in pot and field trials

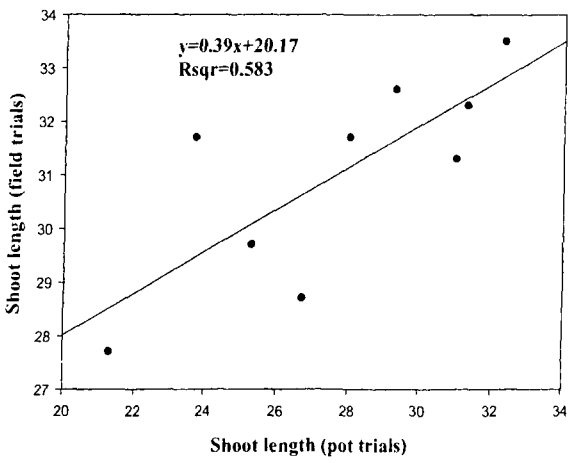

Fig. 63- Linear regression of shoot length of pea grown in pot and field trials

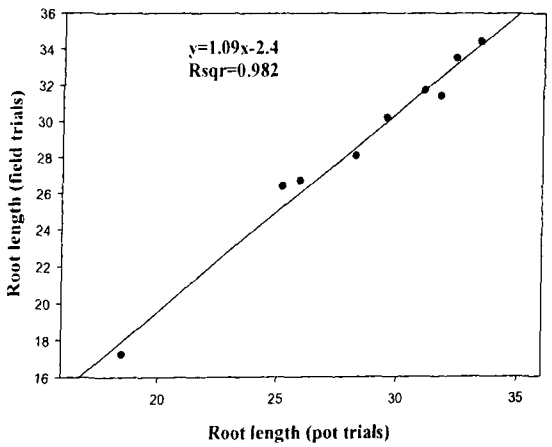

Fig. 64 - Linear regression of root length of greengram grown in pot and field trials

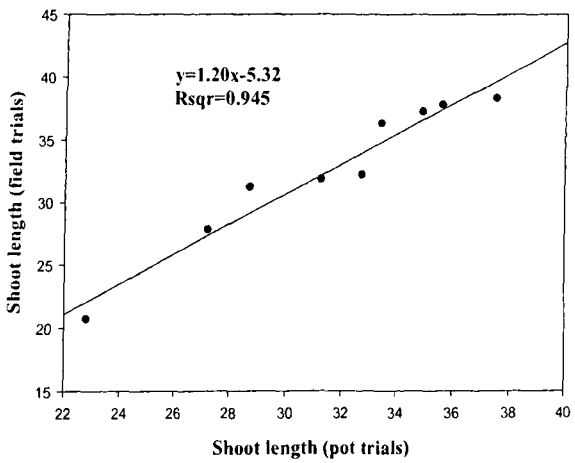

Fig. 65 Linear regression of shoot length of greengram grown in pot and field trials

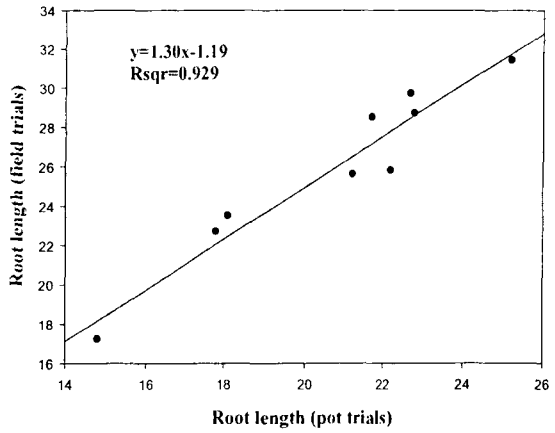

Fig. 66 Linear regression of root length of lentil grown in pot and field trials

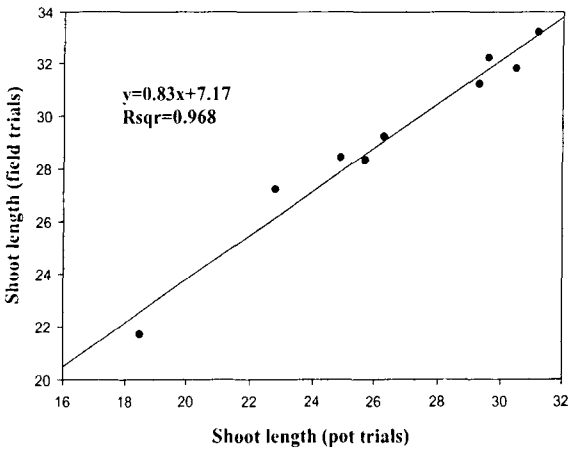

Fig. 67 - Linear regression of shoot length of lentil grown in pot and field trials

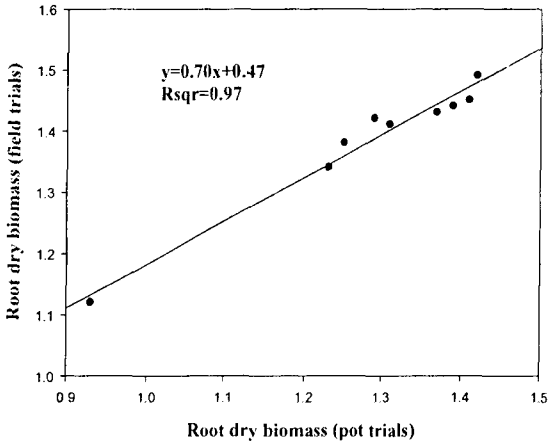

Fig. 68 Linear regression of root dry biomass of chickpea grown in pot and field trials

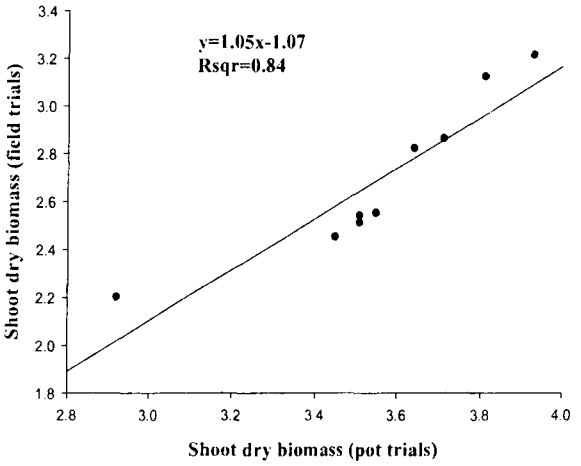

Fig. 69 · Linear regression of shoot dry biomass (g/plant) of chickpea grown in pot and field trials

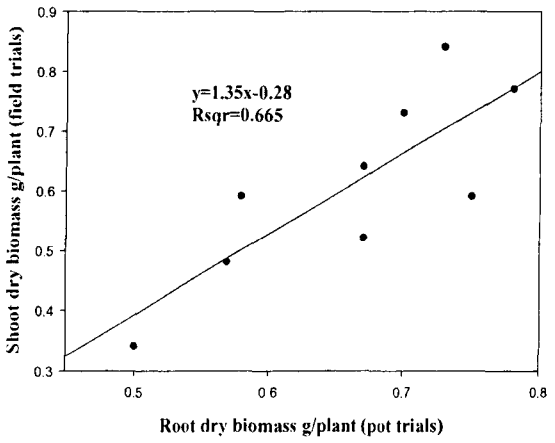

Fig. 70 · Linear regression of root dry biomass (g/plant) of pea grown in pot and field trials

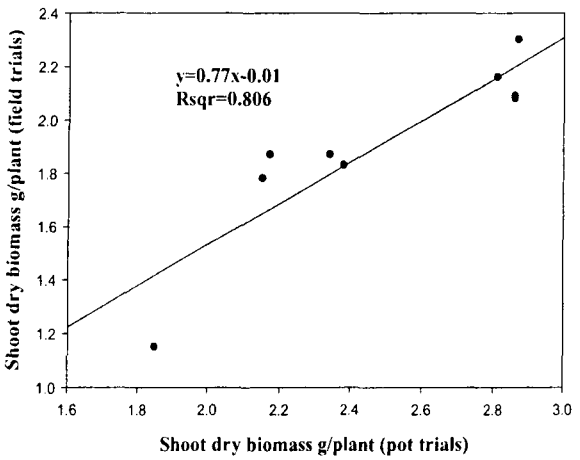

Fig. 71 · Linear regression of shoot dry biomass (g/plant) of pea grown in pot and field trials

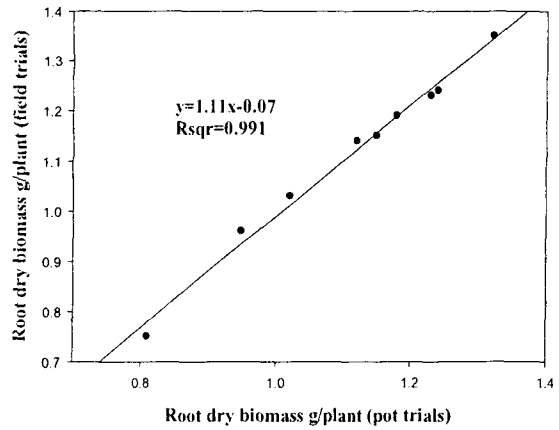

Fig. 72 Linear regression of root dry biomass (g/plant) of greengram grown in pot and field trials

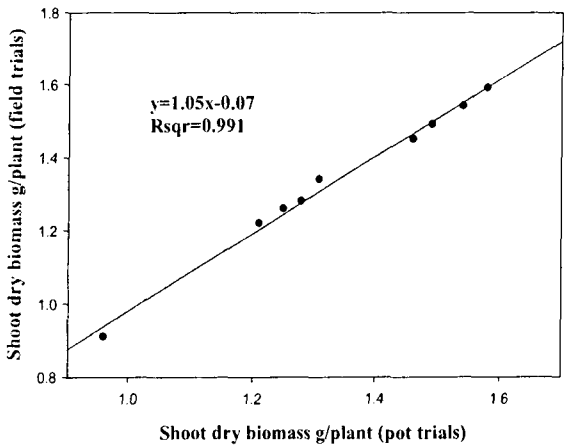

Fig. 73 Linear regression of shoot dry biomass (g/plant) of greengram grown in pot and field trials

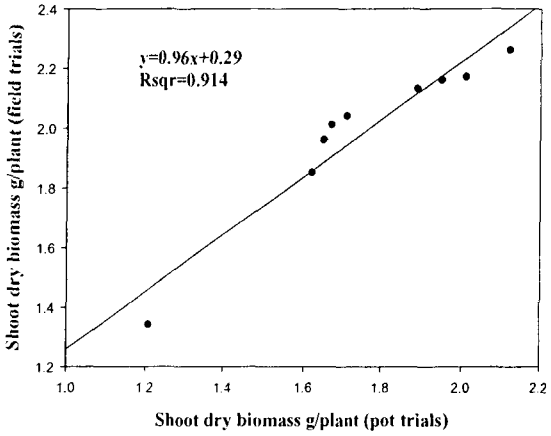

Fig. 74 Linear regression of root dry biomass (g/plant) of lentil grown in pot and field trials

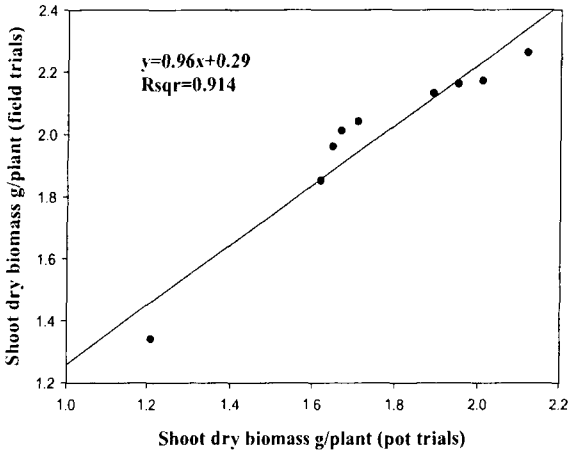

Fig. 75 Linear regression of shoot dry biomass (g/plant) of lentil grown in pot and field trials

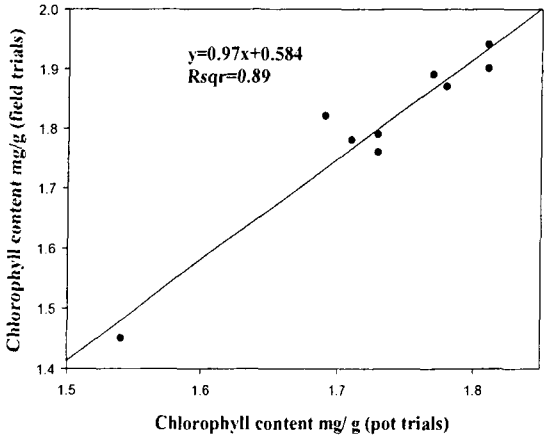

Fig. 76 Linear regression of chlorophyll content (mg/g) of chickpea grown in pot and field trials

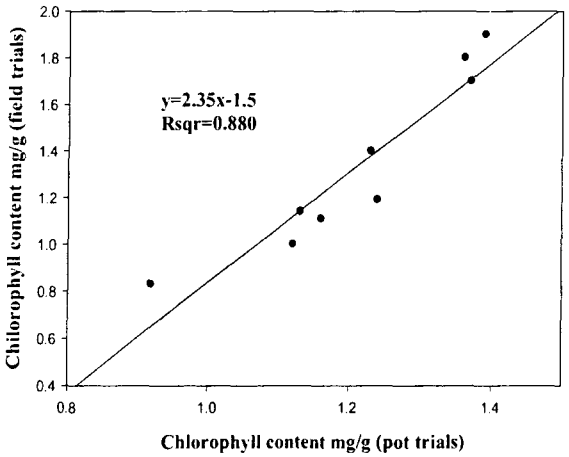

Fig. 77 Linear regression of chlorophyll content (mg/g) of pea grown in pot and field trials

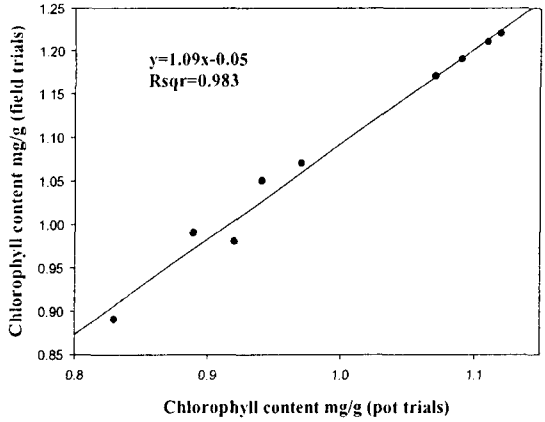

Fig. 78 Linear regression of chlorophyll content (mg/g) of greengram grown in pot and field trials

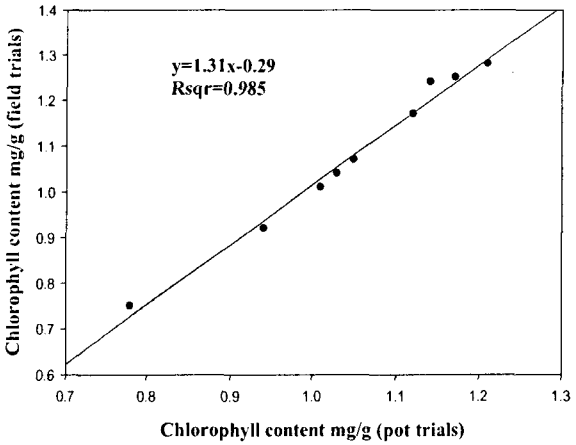

Fig. 79 Linear regression of chlorophyll content (mg/g) of lentil grown in pot and field trials

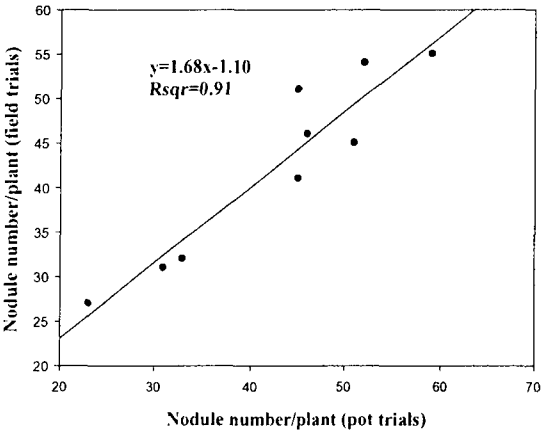

Fig. 80 Linear regression of nodule no./plant of chickpea grown in pot and field trials

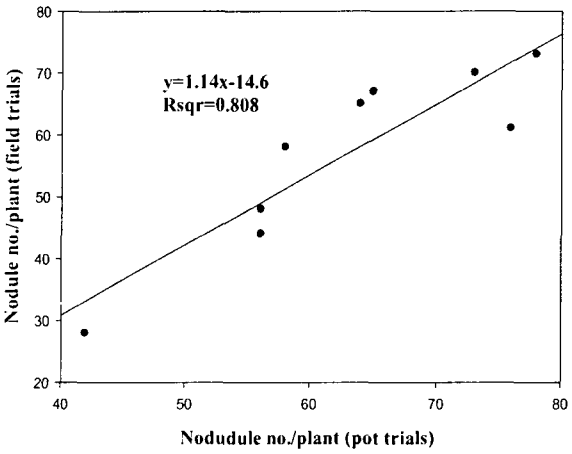

Fig. 81 Linear regression of nodule no./plant of pea grown in pot and field trials

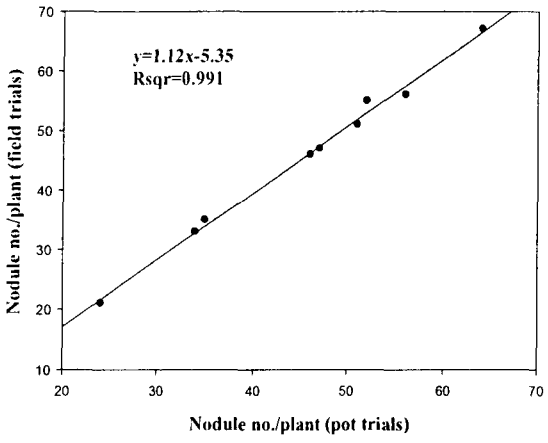

Fig. 82 Linear regression of nodule no./plant of greengram grown in pot and field trials

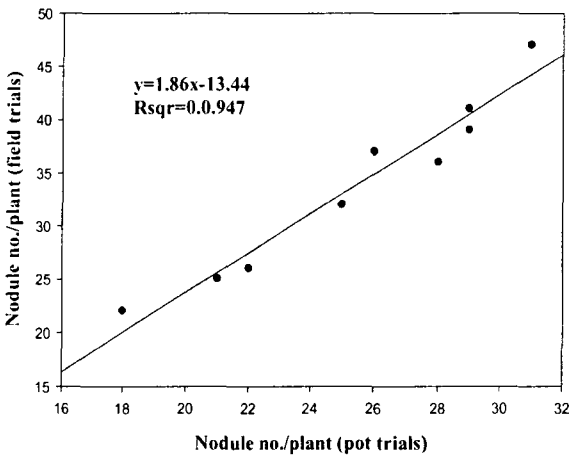

Fig. 83 Linear regression of nodule no./plant of lentil grown in pot and field trials

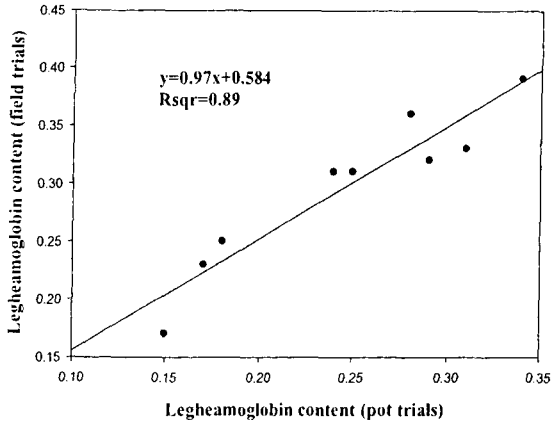

Fig. 84 Linear regression of Lb content of chickpea grown in pot and field trials

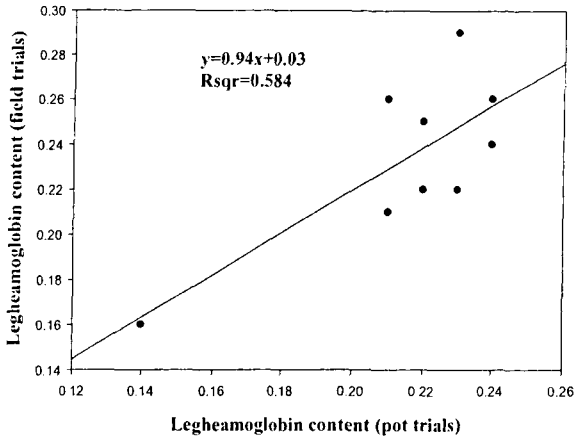

Fig. 85 Linear regression of Lb content of pea grown in pot and field trials

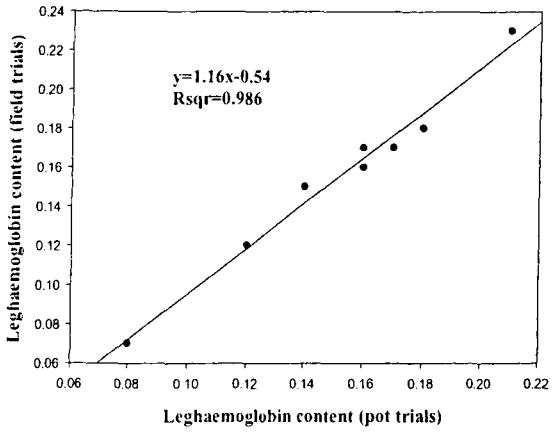

Fig. 86 Linear regression of Lb content of greengram grown in pot and field trials

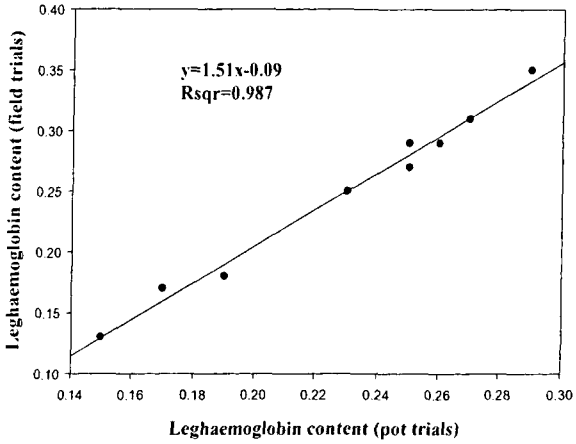

Fig. 87 Linear regression of Lb content of lentil grown in pot and field trials

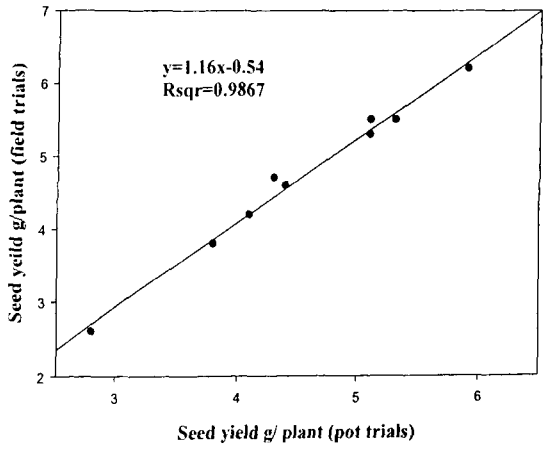

Fig. 88 Linear regression of seed yield (g/plant) of chickpea grown in pot and field trials

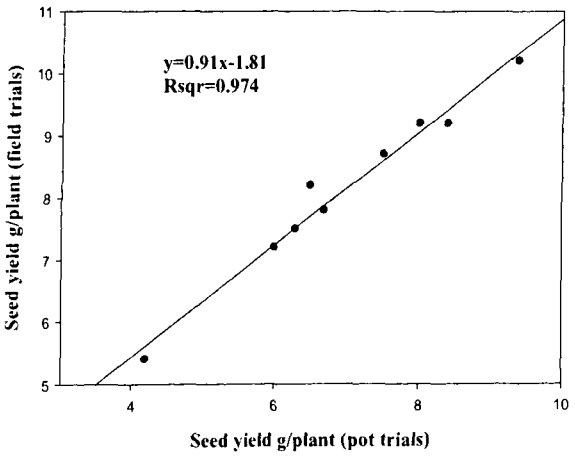

Fig. 89 Linear regression of seed yield (g/plant) of pea grown in pot and field trials

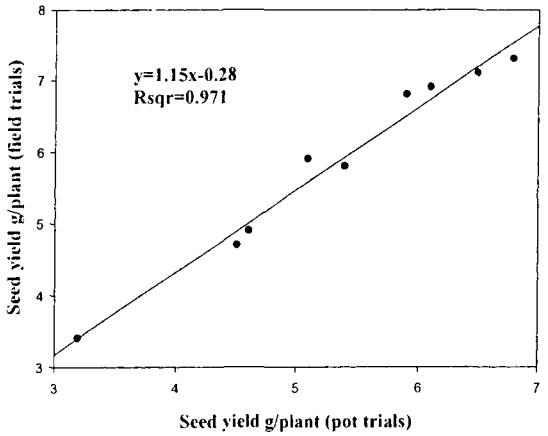

Fig. 90- Linear regression of seed yield (g/plant) of greengram grown in pot and field trials

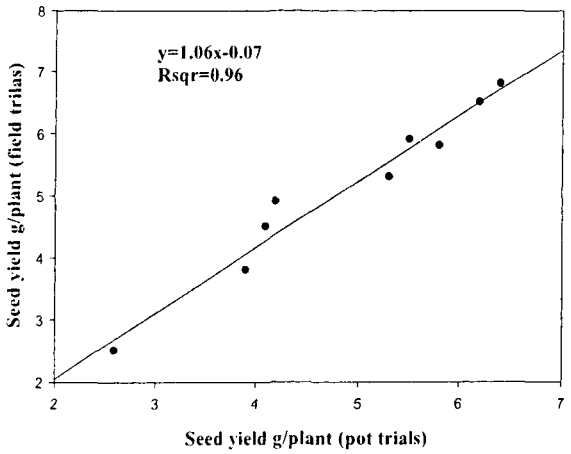

Fig. 92- Linear regression of seed yield (g/plant) of lentil grown in pot and field trials

5.6.5 Seed yield

The plant growth promoting rhizobacteria play an active role in soil through their natural ability to provide important but scarce nutrients to the plants. Among the plant nutrients, N and P are the two key plant nutrients provided by these organisms under natural field conditions. In this context, the inoculation effects of PGPR including N_2 fixers and PSB are receiving increased attention for their use to develop microbial inoculants in order to improve crop productivity. The synergistic effects of N_2 fixer and PSM on plant vigor, nutrient uptake, and yields of various crops have been reported (Zadi et al., 2003; Tilak et al., 2006; Mishra et al., 2013). Considering this, the impact of fertilizers and microbial inoculation on seed of chickpea, pea, greengram and lentil plants was assessed while grown in pot/field soils treated with/without chemical fertilizers. Among fertilizers, DAP when applied alone marginally increased the seed yield of chickpea by 8 (pot) and 11% (field), of pea by 5 (pot) and 4% (field), of greengram by 3 (pot) and 4% (field) and lentil seed by 5 (pot) and 18% (field) at harvest (Table 89). Similarly, the sole application of microbial cultures like *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* specific to each legume showed superior impact than the single application of either urea or DAP on the measured parameters of chickpea, pea, greengram and lentil plants. As an example, *B. pumilus* had an obvious stimulatory effects on seed formation in all legumes and hence, enhanced the seed yield significantly ($P \leq 0.05$) by 13 (pot) and 24% (field), pea seed yield by 8.3 (pot) and 14% (field), greengram seed yield by 13 (pot) and 26% (field) and lentil seed by 36 (pot) and 39% (field) at harvest (Table 89). Furthermore, the dual application of *B. pumilus* and *Rhizobium* specific to each legume was superior over fertilizers (both independent and mixture) and single and

multiple inoculation treatments. For example, *B. pumilus* with *M. ciceri* (chickpea) *B. pumilus* with *R. leguminosarum* (pea) *B. pumilus* with *Bradyrhizobium* (greengram) and *B. pumilus* with *Rhizobium* sp. (lentil) increased the seed yield by 22 (pot) and 16% (field), 13 (pot) and 14% (field), 12 (pot) and 6% (field) and 8 (pot) and 6% (field) over urea with DAP. Generally, the inoculation effects of PGPR used in this study were more profound compared with those of the control plants/fertilizer treated plants, suggesting a synergism among the tested organisms, which together increased the legume growth and consequently the seed yield. Moreover, the impact of composite application of bacterial cultures could probably be due to the additive effect of N_2 fixation, production of plant growth regulators (PGRs), improved mineral uptake, suppression of plant diseases and lower ethylene production by the mixed inocula, as also reported by others (Nascimento et al., 2011b; Krey et al. 2013). While comparing the impact of all treatments on seed yield, the grain yield of pea plants recorded for field trials following inoculation or fertilizer application increased in the order:*P.putida+Rhizobium*>urea+DAP=urea+*P.putida*>DAP+*Rhizobium*>*Rhizobium* >*Pseudomonas*>urea=*DAP*. Seed yield was positively correlated between pot and field grown chickpea (Fig. 88), pea (Fig. 89), greengram (Fig. 90) and lentil (Fig. 91), as showed in Table 90.

Conclusion and future perspective

Conclusion and future perspective

The rapidly increasing costs and inexorable toxicity to foods, water and environment resulting from the indiscriminate and excessive application of fertilizers in agricultural practices has become one of the most undeniable challenges before scientist working in different disciplines. Therefore, to solve such serious problems, it has become extremely important to discover some inexpensive alternative to synthetic fertilizers. The use of natural resources like soil inhabiting beneficial microbial communities especially PGPR opens up a new horizon for better plant productivity besides protecting the agro-ecosystems from hazards of agrochemicals. The safety of the soil environment through the application of soil microbes could become a milestone towards profitable crop productivity. Considering the beneficial impact of microbial communities and inadequate and conflicting reports on the use of microflora in different production systems, this investigation was aimed at identifying some novel plant growth promoting rhizobacteria with multiple qualities. Subsequently, the molecularly characterized and best of the lot in terms of plant growth promoting activities were used to inoculate legumes such as chickpea, pea, greengram and lentil, and the impact was observed both in pot and field soils treated with or without chemical fertilizers. The present study revealed that the mixed inoculations of plant growth promoting rhizobacteria, with exceptional qualities as observed here, especially the N_2 fixers and phosphate solubilizers improved the plant vitality, nutrient concentration and grain quality and showed a dramatic increase in seed yield of legumes both under pot and filed conditions. The multiple growth promoting properties such as ACC deaminase synthesis, release of phytohormones affecting root morphogenesis, production of EPS and cyanogenic compounds besides their inherent ability to transform atmospheric nitrogen into usable form of nitrogen (N_2 fixers) and to make soluble P available to plants (P-solubilizers) might have accounted for superior growth, yield and quality of chickpea, pea, greengram and lentil. Since legumes require a considerable amount of important but scarce plant nutrients, inoculation with such favourably interacting PGPR strains are likely to provide an inexpensive alternative to chemical fertilizers for raising the overall performance of legumes in different production systems. Moreover, this microbial approach if implemented properly is likely to protect soils/soil fertility from the nuisance of chemical fertilizers and in effect to human health across different ecological niches.

However, the viability and sustainability of this technology largely depends on the development and distribution of good quality microbial inoculants to farming communities. This technology therefore, requires far-reaching and steady research efforts to find and exemplify more novel PGPR strains with multiple growth promoting activities for their ultimate application under field conditions. Microbiologists together with soil scientists/agronomists thus have a greater responsibility to the society to find ways and resources as to how the use of soil microflora could be extended to the larger section of the field practitioners so that the dependence on chemical fertilizers for enhancing crop productivity under diverse agro-climatic regions of the world could be reduced, if not eliminated completely. This study further suggests that the soil nutrient pool using microbes can be increased by careful management of existing microbial populations. However, limited success in terms of their wide and regular application in agronomic practices has been achieved so far which could probably be due in part to the unawareness about the performance of microbial cultures among practitioners and their variable activity under natural but fluctuating environments. Therefore, in order to make microbes more attractive, cost-effective and meaningful, in crop productivity in different agro-ecological regions, it is needed to have a comprehensive and consequential understanding of microbial interactions occurring in soil environment. Moreover, how soil and farm management practices influence the processes mediated by soil microbes needs to be elucidated. In this context, some molecular strategies including metagenomics have provided some insight to uncover the structure and functions of microbial communities. Genetic manipulation of both microorganisms and plants for important traits such as N_2 fixation, and plant growth promotion besides producing trait specific mutants could play pivotal roles in deciphering the mechanistic basis and evaluating their contribution to increased nutrient availability in soils. Even some success has been achieved here and there by using molecular tools; there is greater need to develop an area-specific microchips which may be suitable for application in any specific region. If developed with suitable multiple traits, such PGPR can be applied back into the same environment from where they originate. This approach is, therefore, likely to reduce the impact of fluctuating environment on the performance of PSM when used for raising the production of different crops grown in many variable regions across the world.

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Appendix 1

Normal saline solution (g/l)

NaCl 0.7

Appendix 2

Nutrient agar (g/l)

Beef extract 3; peptone 5; agar 15; pH 7

Appendix 3

Martin's medium (g/l)

Dextrose 5; potassium dihydrogen ortho-phosphate 1; magnesium sulphate 0.5; streptomycin 0.006; rose Bengal 2 part in 3000 part of medium.

(1g of chloramphenicol/nalidixic acid was dissolved in 100 ml of sterile water. 0.3 ml of this solution was added to 100 ml of rose Bengal medium after it cooled to 45^0C).

Appendix 4

Kenknight's medium (g/l)

Dextrose 1; Potassium dihydrogen phosohate 0.1; Sodium nitrate 0.1; Poassium chloride 0.1;

Magnesium sulfate 1.50

Appendix 5

Pikovskaya medium (g/l)

Glucose 10; $Ca_3(PO_4)_2$ 5; $(NH_4)_2SO_4$ 0.5; NaCl 0.2; $MgSO_4.7H_2O$ 0.1; KCl 0.1; yeast extract 0.5; $MnSO_4$ and $FeSO_4$ trace; pH 7

Appendix 6

Ashby's mannitol agar (g/l)

Mannitol 20.0; Dipotassium hydrogen orthophosphate 0.2; Potassium sulphate 0.1; $MgSO_4$ 0.2; $CaCO_3$ 5.0; NaCl 0.2

Appendix 7

Yeast extract mannitol medium (g/l)

Mannitol 10; K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 0.1; yeast extract 1.0 ; $CaCO_3$ 2; pH 7

Appendix 8

Gram staining

Primary stain*: *Solution A- Crystal violet (90% dye content) 2 g; Ethyl alcohol (95%) 20 ml,

Solution B- Ammonium oxalate 0.8 g; distilled water 80 ml

Gram's iodine

Iodine 1 g; potassium iodide 2 g; distilled water 300 ml

Decolorizer

Ethyl alcohol 95 ml; distilled water 5 ml

Counter stain

Safranin (2.5% solution in 95% ethyl alcohol) 10 ml; distilled water 100 ml

Appendix 9

Nutrient broth (g/l)

Beef extract 3; peptone 5; pH 7

Appendix 10

Kovac's reagent

p-dimethyl amino benzaldehyde 10 g; Iso-amyl alcohol 15 ml

(Dilute 10 times in distilled water before use)

Appendix 11

Simmons citrate agar (g/l)

Ammonium dihydrogen phosphate 1; dipotassium phosphate 1; magnesium sulfate 0.2; sodium chloride 5; sodium citrate 2; bromothymol blue 0.08; pH 7.2

Appendix 12

MR-VP broth (g/l)

Peptone 7; dextrose 5; potassium phosphate 5; pH 6.9

Appendix 13

Methyl red solution (g/l)

Methyl red 0.1; ethyl alcohol 300 ml; distilled water 200 ml

Appendix 14

Trypticase nitrate broth (g/l)

Trypticase 20; disodium phosphate 2; dextrose 1; potassium nitrate 1; agar 20; Ph 7

Solution A (g/l)

Sulfanilic acid 8; acetic acid 5N 1000 ml

(5N: 1 part glacial acetic acid to 2-5 parts distilled water)

Solution B (g/l)

Dimethyl amine 1- napthylamine 5; acetic acid 1000 ml

Appendix 15

Barrit's reagent (g/l)

Solution A

A- naphthol 5; ethanol 95 ml

Solution B

Creatine 0.30; potassium hydroxide 40

Appendix 16

Starch agar (g/l)

Peptone 5; beef extract 3; starch 2; agar 20; pH 7.0

Appendix 17

Fermentation broth (g/l)

Beef extract 1; peptone 10; phenol red 0.018; pH 7.4

Appendix 18

Tributyrin agar (g/l)

Beef extract 3; peptone 5; tributyrin 10; agar 15; pH 7.2

Appendix 19

Minimal salt agar medium (g/l)

KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4.7H_2O$ 0.2; $CaCl_2.2H_2O$ 0.02; $FeSO_4.7H2O$ 0.01; pH 6.5

Appendix 20

DF salt medium (g/l)

KH_2PO_4 4; Na_2HPO_4 6, $MgSO_4.7H_2O$ 0.2, Glucose 2.0, Gluconic Acid 2.0; Citric Acid 2.0; trace elements 1 mg $FeSO_4.7H_2O$, 10 µg H_3BO_3, 11.19 µg $MnSO_4.H_2O$, 124.6 µg $ZnSO_4.7H_2O$, 78.22 µg $CuSO_4.5H_2O$, 10 µg MoO_3, pH 7.2 and 2.0 g $(NH_4)_2SO_4$ as nitrogen source.

Appendix 21

Chloromolybdic acid

Ammonium molybdate 15 g; distilled water 400 ml; 10 N HCl 400 ml

The above described materials were mixed slowly with rapid stirring, cool and make the volume to 1 liter with distilled water

Appendix 22

Chlorostannous acid

Stannous chloride 10 g; concentrated hydrogen chloride 25 ml

The stock solution was kept in air tight bottle. 1ml of stock solution is mixed in 132 ml of distilled water at the time of experiment.

Appendix 23

Luria Bertani (LB) broth (g/l)

Tryptone 10; yeast extract 5; Nacl 10; pH 7.5

Appendix 24

Chrome Azurol S (CAS) agar medium

CAS agar is prepared from four solutions

Solution 1: Fe-CAS indicator solution

Mix 10 ml of 1 mM $FeCl_3.6H_2O$ [in 10 Mm HCl} with 50 ml of an aqueous solution of CAS (1.21 mg/ml). The above solution was then added to 40 ml of HDTMA (1.82 mg/ml) and cooled to 50 ^{0}C.

Solution 2: Buffer solution

Dissolve 30.24 g of PIPES in 750 ml of a salt solution containing 0.3 g KH_2PO_4, 0.5 g NaCl and 1 g NH_4Cl, pH 6.8 with 50% KOH and water was added to bring the volume to 800 ml.

Solution 3: in 70 ml water

2 g glucose, 2 g mannitol, 493 mg $MgSO_4$. $7H_2O$, 11 mg $CaCl_2$, 1.17 mg $MnSO_4$. H_2O, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4$. $5H_2O$, 1.2 mg $ZnSO_4$. $7H_2O$ and 1 mg Na_2MoO_4. $2H_2O$. Autoclaved , cooled to 50 ^{0}C, then added to the buffer solution along with 30 ml filter-sterilized 10% (W: V) casamino acids (solution 4). The indicator solution was added last with sufficient stirring to mix the ingredients without forming bubbles.

Appendix 25

Modi medium

K_2 HPO_4 0.05%; $MgSO_4$ 0.04%; NaCl 0.01%; mannitol 1%; glutamine 0.1%; NH_4NO_3 0.1%

Appendix 26

HCN induction medium (g/l)

Tryptic soy broth 30; glycine 4.4; agar 15

Appendix 27

Peptone water (g/l)

Peptone 10; NaCl 5; pH 7

Appendix 28

Nessler's reagent

Potassium iodide 50 g; distilled water (ammonia free) 35 ml

Add saturated aqueous solution of mercuric chloride until a slight precipitate persists

Potassium hydroxide 400 ml

Dilute the solution to 1000 ml with ammonia free distilled water. Allow to stand for one week, decant supernatant liquid and store in a tightly capped amber bottle.

Appendix 29

Potato dextrose agar (g/l)

Potato infusion form 4; Dextrose 20; Agar 15; pH 5.4

Appendix 30

Phosphate buffer 1% (pH 7.2-7.4)

Solution A- Disodium phosphate 1.4 g; distilled water 100 ml

Solution B-Sodium dihydrogen phosphate 1.4 g; distilled water 100 ml

(84.1 ml of solution A to 15.9 ml of solution B and 8.5 g of sodium chloride and volume was made upto one liter)

Appendix 31

Pyridine reagent

Sodium hydroxide 0.8 g (dissolved in 50 ml), pyridine 33.8 ml. The volume was made upto 100 ml

Appendix 32

Copper solution

Solution A: Sodium carbonate 2g (mixed with 0.1 N NaOH)

Solution B: Copper sulphate 0.5 g, potassium sodium tartrate 1g, distilled water 100 ml

Copper solution was prepared by mixing 50 ml solution A with 1 ml of solution B

Appendix 33

Folin's reagent

Sodium tungstate 100 g, sodium molybdate 25 g, distilled water 700 ml, 85% ortho-phosphoric acid 50 ml, HCl 100 ml, bromine water few drops (Reflux the above given mixture for 10 h). Boil the solution without condenser for 15 min. to remove excess bromine, cool and dilute it to 1 liter

List of Publications

Ahmad E, Khan MS, Zaidi A (2013) ACC deaminase producing *Pseudomonas putida* strain PSE3 and *Rhizobium leguminosarum* strain RP2 in synergism improves growth, nodulation and yield of pea grown in alluvial soils. Symbiosis, 61:93–104

Khan MS, **Ahmad E**, Zaidi A, Oves M (2013) Functional Aspect of Phosphate-Solubilizing Bacteria: Importance in Crop Production, In: Maheshwari et al. (eds), Bacteria in Agrobiology: Crop Productivity, Springer-Verlag Berlin Heidelberg, pp 237-263

Ahmad E, Zaidi A, Khan MS, Oves M (2012) Heavy Metal Toxicity to Symbiotic Nitrogen Fixing Microorganism and Host Legumes. In: A. Zaidi et al. (eds.), Toxicity of Heavy Metals to Legumes and Bioremediation, pp 29-44

Oves M, Khan MS, Zaidi A, **Ahmad E** (2012) Soil contamination, nutrientive value and human risk assessment of heavy metals: An overview. In: A. Zaidi et al. (eds.), Toxicity of Heavy Metals to Legumes and Bioremediation, pp 29-44

Zaidi A, Oves M, **Ahmad E**, Khan MS (2011) Importance of free-living fungi in heavy metal remediation. In: MS Khan, Zaidi A, Goel R, Musarrat J(eds.), *Biomanagement of Metal-Contaminated Soils*, Environmental Pollution 20, Springer, The Netherlands

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SUMMARY

In high input agricultural practices huge quantities of agro-chemicals including both fertilizers and pesticides are applied regularly but injudiciously in order to accomplish maximum crop production. Of these, relatively higher amounts of unutilized fertilizers persists in soil and may cause toxicity to soil microflora/fauna, waters and consequently foods and via different food chains to human health. Therefore, the sustainability in agricultural systems without compromising the environmental quality and conservation has become one of the major concerns around the world. So, due to the spiraling costs and severe toxicity to foods, water and environment resulting from the indiscriminate application of fertilizers it has become even more imperative to discover some inexpensive alternative to meet out such challenges. In this context, plant growth promoting rhizobacteria indeed has provided some solutions to the problems. Considering the beneficial impact of microbial communities and inadequate and conflicting reports available on the use of microflora in different production systems, this investigation was aimed at identifying some novel plant growth promoting rhizobacteria with multiple qualities. Subsequently, the molecularly characterized and best of the lot in terms of plant growth promoting activities were used to inoculate legumes such as chickpea, pea, greengram and lentil, and the impact was observed both in pot and field soils treated with or without synthetic fertilizers. To achieve these, the present investigation was therefore, designed with specific following objectives:-

(i) to assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area

(ii) isolation of N_2 fixing bacteria from the nodules of legumes grown in conventional soils and P- solubilizing bacteria from different rhizospheric soils

(iii) to isolate ACC deaminase producing bacterial strains from different rhizospheres

(iv) to assay the production of plant growth promoting substances by the PGPR strains both qualitatively and quantitatively

(v) to characterize the PGPR strains morphologically, biochemically and by 16S rRNA gene sequence analysis

(vi) assessment of the impact of microbial inoculants on the performance of chickpea, pea, greengram and lentil grown in sandy clay loam soils treated with/without nitrogenous/phosphatic fertilizers and

(vii) nutrient uptake analysis in the test legumes

The rhizospheric soils of mentha, chilli, cabbage, mustard, chickpea, pea, greengram, and lentil, grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were used to determine microbial diversity. The viable counts of bacteria, fungi and actinomycetes differed considerably among rhizosphere soils. Generally, the total bacterial populations was highest ($4.28x10^7$ cfu/g soil) while those of actinomycetes was lowest ($1.6x10^4$ cfu/g soil) in all soil samples tested. The order of microbial population in all soil samples was found as: bacteria>fungi>actinomycetes. Among different rhizospheres, the bacterial populations was recorded lowest ($3.42x10^7$ cfu/g soil) in cabbage rhizosphere while in chickpea, pea, greengram and lentil it was $3.62x10^7$, $2.71x10^7$, $4.21x10^7$ and $3.94x10^7$ cfu/g soil, respectively. The rhizospheric soils of mentha, however, showed a considerable increase of 21, 25, and 11% in bacterial populations compared to those recorded for chilli, cabbage, and mustard, respectively. The fungal populations in all the rhizospheric soils ranged from $1.1x10^5$ (lentil) to $1.8x10^5$ (mentha) cfu/g soil. The populations of asymbiotic N_2 fixer (ANF) varied noticeably among rhizosphere soils. The ANF in rhizospheric soils ranged between $1.9x10^5$ cfu/g soil (mustard) to $3.2x10^5$cfu/g soil (pea). Moreover, the populations of PSB were greater (mean value $5.24x10^5$ cfu/g soil) in all samples than theP S fungi ($5.20x10^3$ cfu/g) . Similarly, the PSF counts were recorded highest in pea ($6.8x10^3$ cfu/g soil) and lowest in mentha ($3.2x10^3$ cfu/g) rhizospheric soils. While comparing the PSM (including bacteria and fungi) populations in all the rhizosphere soils, the order was: greengram>pea>mentha>chickpea>lentil>chilli>cabbage> mustard. Furthermore, the isolated bacterial cultures showed a variable morphological and biochemical characteristics. Generally, the rhizobial strains were Gram negative while PSB showed a variable Gram reaction. Rhizobial strains in general were positive to all the biochemical reactions except methyl red, Voges Proskauer, indole and gelatin hydrolysis test. In contrast, the PSB showed a considerable variation in biochemical properties. Among the bacterial strains, 38% each of *Mesorhizobium* spp. (chickpea) and *Rhizobium* spp. (pea), 33% each of *Bradyrhizobium* spp. (greengram) and *Rhizobium* spp. (lentil), 40% *Azotobacter* spp. and 36% of PSB were tested further for

evaluating the synthesis of ACC deaminase, phosphate solubilization, IAA, production of siderophores, ammonia, hydrogen cyanide and EPS and antifungal activity. Based on the PGP activities observed under *in vitro* conditions, the mesorhizobial strains were grouped into four PGP groups. All strains of *Mesorhizobium* produced IAA, NH_3 and EPS while 67% strains showed ACC deaminase activity. A total of 47% mesorhizobial strains had both siderophore and HCN activity. Of these, 13% demonstrated both P-solubilization and antifungal activity. The PGP group I included one strain (RG5) which showed 8 PGP traits followed by group II, which had only one strain (RG4) positive to ACC deaminase, IAA, siderophore, NH_3, HCN, EPS and antifungal activity. In PGP group III, 5 strains exhibited a positive reaction to ACC deaminase, IAA, siderophore, NH_3 and EPS, while PGP group IV had 3 bacterial strains showing positive reaction to ACC deaminase, IAA, NH_3, and EPS. The PGP group V, had only one strain (RG6) which showed P- solubilization, IAA, synthesize NH_3 and EPS while PGP group VI included four strains positive for IAA, NH_3, and EPS. All *Rhizobium* strains isolated from pea nodules produced IAA, NH_3 (100%) and EPS where as only 47% strain could synthesize ACC deaminase and HCN. Siderophores, antifungal activity and P-solubilizing activity was shown by 33, 40, and 13% strains, respectively. Similarly, *Rhizobium* strains isolated from pea nodules were grouped into three PGP groups. The PGP group I included two strains (RP2 and RP6) with 7 PGP followed by PGP group II, which had 3 strains positive to ACC deaminase, IAA, siderophore, NH_3, HCN, and EPS. Two strains in PGP group III, displayed a positive reaction to ACC deaminase, IAA, synthesis of NH_3, EPS and antifungal activity while group IV included only one strains capable of secreting IAA, NH_3, HCN and EPS. The PGP group V included 6 strains which synthesized IAA, NH_3 and EPS. Interestingly, all strains of *Bradyrhizobium* were able to synthesize NH_3 and EPS while IAA was produced by 90% strains. Siderophores, HCN, ACC deaminase activity, P-solubilization, and antifungal activity were shown by 50, 50, 20, 10 and 30%, respectively. Similarly, other PGPR were divided into different functional groups.

Rhizobia including *Mesorhizobium* (chickpea nodules), strains of *Rhizobium* (pea nodules), *Bradyrhizobium* (greengram nodules), and *Rhizobium* (lentil nodules) and P-solubilizing bacteria were positive for ACC deaminase activity. The ACC deaminase activity among *Mesorhizobium* ranged from 113 µmol α-ketobutyrate/mg protein/h (RG8) to 258 µmol α ketobutyrate/mg protein/h (RG4) while among *Rhizobium* it

differed between 132 µmol α ketobutyrate/mg protein/h (RP10) to 238 µmol α-ketobutyrate/mg protein/h (RP2). *Bradyrhizobium*, and *Rhizobium* isolated from greengram and lentil nodules, respectively were positive to ACC deaminase. strain PSE9 of PSB produced 227 µmol α ketobutyrate/mg protein/h while strain PSE3 could synthesize 625 µmol α ketobutyrate/mg protein/h. *Achromobacter* sp. ES1 produced 163 µmol α ketobutyrate/mg protein/h) and *Pseudoxanthomonas* sp. strain ES5 (578 µmol α ketobutyrate/mg protein/h), respectively. Moreover, a total of 14% rhizobacterial strains showed PS activity and produced halo on solid Pikovskaya which ranged from 6 mm (*Rhizobium* sp. RP6) to 10 mm (*Mesorhizobium* sp. RG5). The SI value for rhizobia ranged from 1.4 (*Bradyrhizobium* sp. RB9) to 2.1 (*Bradyrhizobium* sp. RB6) while for *Azotobacter* spp. it was 1.4 (AZ 20) to 2.6 (AZ 19) and for P-solubilizing bacteria the S.I. varied between 1.5 (*Bacillus* sp. PSE16) to 3.8 (*P. putida* PSE5). The solubilizing efficiency (S.E.) of each P-solubilizer differed from 42 (*Bradyrhizobium* sp. RB9) to 116 (*Bradyrhizobium* sp. RB6) for rhizobia. 50 (*Azotobacter* AZ20) to 150 (*Azotobacter* AZ5) for *Azotobacter* spp. and 50 (*Enterobacter* PSE15) to 333 (*Pseudomonas* PSE3), respectively. The amount of P-solubilized by rhizobia ranged from 45 µg/ml (*Rhizobium* sp. RP6) to 148 µg/ml (*Bradyrhizobium* sp. RB6), 87 µg/ml (*Azotobacter* sp. AZ20) to 215 µg/ml (*Azotobacter* sp. AZ19) among non-symbiotic N_2 fixers and 111 µg/ml (*Enterobacter* sp. PSE26) to 321 µg/ml (*Achromobacter* PSE28). In addition, the solubilization of TCP by different bacterial cultures was coupled with consequent decrease in pH values that ranged between 5.7 (*Rhizobium* sp. RV9) to 6.1 (*Mesorhizobium* sp. RG6) , 5.2 (*Azotobacter* AZ10 and *Azotobacter* AZ19) to 5.9 (*Azotobacter* AZ1) and 4.4 (*Bacillus* PSE21) to 5.8 (*Enterobacter* PSE30).

The production of IAA by the selected bacterial genera assayed in LB broth treated with (100 µg/ml) or without (0 µg/ml) tryptophan varied among treatments. The amount of IAA synthesized by mesorhizobial strains varied between 14 (RG14) to 29 µg /ml (RG10) in LB broth without tryptophan and 32 (RG14) to 75 µg /ml (RG4) in LB broth supplemented with 100 µg/ml tryptophan. Among the pea specific *Rhizobium* isolates, strain RP9 produced a maximum amount of 32 (0) and 73 µg/ml IAA (100 µg tryptophan/ml). The amount of IAA synthesized by rhizobial strains varied between 13 (RP15) to 32 µg /ml (RP9) at 0 µg/ml tryptophan and 41 (RP3) to 75 µg /ml (RP8) at 100 µg/ml tryptophan, respectively. *Bradyrhizobium* strains also

produced a significant amount of IAA, maximum being 95 µg/ml IAA by the strain RB4 followed by 85 µg/ml IAA at 100 µg/ml tryptophan, . Similarly, the *Rhizobium* strains isolated from lentil nodules showed a variable amount of IAA. Of the *Azotobacter* sp., strains AZ19 and AZ4 were most effective and produced 96 and 89 µg/ml IAA at 100 µg/ml tryptophan, respectively. Among P-solubilizers, PSE25 maximally produced IAA (62 µg/ml) which was followed by PSE24 (62 µg/ml IAA). Generally, the synthesis of IAA by all molecularly characterized P-solubilizers was greater when grown in medium treated with tryptophan than those recorded for untreated medium. In yet other study, the production of IAA was increased with increasing concentration of tryptophan but there were little difference in the synthesis of IAA between the incubation intervals among rhizobial strains. On CAS agar plates, a total of 47% of the *Mesorhizobium* strains produced a visible orange yellow halo on CAS agar plates after five days of incubation whose size varied between 10 (*Mesorhizobium* RG1) to 12 mm (*Mesorhizobium* RG3 and RG8). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain RG8 yielded 16 and 33 µg/ml of 2,3-dihydroxy benzoic acid (DHBA) and salicylate (SA), strain RG7 produced 15 and 34 µg/ml of DHBA and SA, strain RG5 yielded 13 and 28 µg/ml of DHBA and SA, and strain RG4 produced 15 and 29 µg/ml of DHBA and SA, respectively. Similarly, 33% of the pea rhizobia showed an orange yellow colored zone which was greater than 10 mm in size. Additionally, among the siderophore positive rhizobial strains, strain RP6 maximally enhanced the DHBA by 25% relative to the poorly DHBA secreting strain RP2 while strain RP10 showed maximum increase in SA (63%) compared to ~~the~~ lowest SA producing strain RP3. Similarly, strains of *Bradyrhizobium* species showed orange yellow colored zone of varying sizes after five days of incubation. Strain RB3 considerably enhanced DHBA by 14% while it increased the SA by 40% in comparison to the lowest siderophore synthesizing strain RB6. In a similar manner, *Rhizobium* species isolated from lentil nodules, *Azotobacter*, *Achromobacter*, *P. putida*, *Enterobacter*, *B. pumilus*, *Pseudoxanthomonas*, and *Stenotrophomonas* showed variable amounts of siderophores. Additionally, the strains of mesorhizobium, rhizobia, *Bradyrhizobium*, *Azotobacter* and PSB were positive to EPS, NH_3 and cyanogenic compounds.

Antifungal activity of N_2-fixers (N=70) and P-solubilizers (N=30) assessed on PDA differed considerably against three phytopathogens, namely, *Rhizoctonia* sp.,

Penicillium sp. and *Alternaria* sp. Also, *Azotobacter* sp., and few strains of P-solubilizers inhibited the growth of test phytopathogens. The sensitivity/resistance profile of N_2-fixers and P-solubilizers determined using disc diffusion method was variable. On the basis of molecular characteristics, some of the bacterial strains were identified as *Pseudomonas putida* strain PSE3 and PSE5 (Gene Bank accession number HM236047 and HM236047), *Achromobacter* strain ES1 and ES6 (Gene Bank accession number JX483710 and JX 965905), *Enterobacter* strain ES2 (Gene Bank accession number JX 965901) *Bacillus pumilus* strain ES3 (Gene Bank accession number JX 965902), *Pseudoxanthomonas* strain ES4 (Gene Bank accession number JX 965903) and *Stenotrophomonas* strain ES5 (Gene Bank accession number JX 965904). Later on, phylogenetic tree of eight P-solubilizer strains was constructed.

Considering the importance of soil microbes in enhancing the crop production, and expression of multiple growth promoting activities by PGPR strains as observed here, some of the potential PGPR strains were further used to assess their impact on chickpea, pea, greengram and lentil grown under both pot and field soils treated with/without recommended rates of urea and diammonium phosphate (DAP). The recommended rates of urea and DAP in general, did not have any significant ($P \leq 0.05$) effect on the biological and chemical properties of chickpea, pea, greengram and lentil grown in alluvial soils compared to those of single or composite application of microbial cultures. Of the two fertilizers, DAP showed a profound impact on the measured parameters of the legumes. For example, *P. putida* among sole inoculation, had an obvious stimulatory effects on dry matter accumulation in all legumes and enhanced the total dry biomass of chickpea by 8 (pot) and 7% (field), pea dry matter yield by 12 (pot) and 8% (field), greengram biomass by 15 (pot) and 17% (field) and lentil biomass by 8 (pot) and 13% (field) at harvest over DAP. Microbial cultures in the presence of recommended rates of urea and DAP further increased the whole biomass of each legume. The co-culture of *P. putida, Bacillus, Azotobacter* and [*M. ciceri* (chickpea)], [*R. leguminosarum* (pea)], *Bradyrhizobium* sp. (vigna) and *Rhizobium* sp. (lentil) strains showed a more profound impact on biological and chemical characteristics of chickpea, pea, greengram and lentils. The P-solubilizers (*P. putida*, *B. pumilus* and *Azotobacter*) when used in association with N-fixers (rhizobia, *Mesorhizobium/Bradyrhizobium*) had the most identifiable effects and tremendously increased the chlorophyll contents of each legume relative to other inoculated/uninoculated plants grown in soils treated with/without fertilizers. As an

example, pea plants co-inoculated with [*B. pumilus* with *R. leguminosarum*] had the highest chlorophyll content in foliage of both pot and field grown plants compared to uninoculated and untreated control. Similarly, the co-cultures of *Bradyrhizobium* with [*P. putida*], [*B. pumilus*] and [*Azotobacter*] maximally increased the chlorophyll content in fresh foliage of greengram by 35, 37 and 33%, respectively as compared to control plants grown in pot while the chlorophyll content in field crops was increased by 37, 39 and 34%, respectively. The symbiotic characteristics (nodulation and leghaemoglobin content) of inoculated/uninoculated chickpea, pea, greengram and lentil plants grown in soils treated with or without urea/DAP was variable. Among fertilizer, DAP in general showed increasing effect on nodulation and leghaemoglobin over urea but it was statistically non significant ($P \leq 0.05$). The sole application of rhizobia specific to each legume in contrast remarkably increased the symbiotic characteristics relative to other single microbial cultures or sole application of urea/DAP, both in pot and fields. As an example, rhizobia when used alone, significantly enhanced the nodulation in (i) chickpea grown in pot (58%) and field (69%) (ii) pea by 14 (pot) and 35% (field) (iii) greengram 49 (pot) and 57% (field) and (iv) lentil by 27 (pot) and 38% (field) over single application of DAP. The leghaemoglobin content in each rhizobia inoculated legumes were higher than those recorded for fertilizer treated/other culture treatments. The co-culture of *B. pumilus* with rhizobia in particular performed exceptionally well and enhanced the nodulation and leghaemoglobin content profoundly compared to other microbial or fertilizer treatments . For example, the composite inoculation of [*Rhizobium* with *B. pumilus*] significantly increased the NN, NDB and Lb content in fresh nodules by 86, 91 and 86% (pot experiment) and by 169, 95 and 94% in field grown peas above the control at 90 DAS, respectively. Inoculation of legumes with PSB and N_2 fixers (both rhizobia and *Azotobacter*) considerably increased the N and P accumulation within roots and shoots of chickpea, pea, greengram and lentil plants grown in soils treated with/without chemical fertilizers. Moreover, the combined inoculation effects were greater than the sum of the individual inoculation effects, suggesting synergism beyond simple additive effects (positive multiplicative interaction). For example, the highest increase in concentrations of N and P was recorded with *B. pumilus* with *Bradyrhizobium* in root and shoots of greengram plants over DAP application. In contrast, the application of *Azotobacter* with rhizobia in general had a poor impact on N and P contents of both roots and shoots of all legumes. *Pseudomonas putida*, *B.*

pumilus, *Azotobacter* and *Rhizobium* when used alone, significantly ($P \leq 0.05$) enhanced the N contents in roots and shoots of field grown lentil plants by 38 and 56%, 43 and 61%, 29 and 54% and, 52 and 72%, respectively relative to control . The P concentration in roots and shoots of lentil plants grown in pots following sole application of *P. putida* and *B. pumilus*, *Azotobacter* and *Rhizobium* was massively increased by 52 and 48%, 64 and 58%, 36 and 42% and, 64 and 55%, respectively, over control. The co-culture of [*Rhizobium* and *B. pumilus*] markedly augmented the N concentration in roots and shoots by 105 and 76% (pots) and 86, 95% (field) while P content in roots and shoots of pot grown lentil was enhanced by 104 and 77% and in field grown plants it was 104 and 76% above pot/field control plants. The impact of mixture of both urea (30 kg/ha) and DAP (90 kg/ha) on the measured parameters was statistically significant compared to other single treatment of urea or DAP or control plants . Of the two fertilizers, 90 kg DAP/ha in general, produced maximum positive effect on the measured parameters of either inoculated or un-inoculated lentil plants.

The impact of fertilizers and microbial inoculations on both quantity and quality of chickpea, pea, greengram and lentil grains was assessed in pot/field experiments. There were no significant difference among the two fertilizers in terms of seed yield or grain protein of chickpea, pea, greengram and lentil at harvest. In contrast, the sole application of microbial cultures like *P. putida*, *B. pumilus*, *Azotobacter* and *Rhizobium* specific to each legume exhibited superior impact over single application of either urea or DAP on the measured parameters. As an example, *B. pumilus* enhanced the seed yield by 13 (pot) and 24% (field), pea yield by 8.3 (pot) and 14% (field), greengram yield by 13 (pot) and 26% (field) and lentil seed by 36 (pot) and 39% (field) at harvest. The seed yield was further enhanced due to dual inoculation of *B. pumilus* and *Rhizobium* specific to each legume compared to fertilizers (both independent and mixture) and single and other multiple inoculation treatments. For example, *B. pumilus* with *M. ciceri* (chickpea) *B. pumilus* with *R. leguminosarum* (pea) *B. pumilus* with *Bradyrhizobium* (greengram) and *B. pumilus* with *Rhizobium* sp. (lentil) increased the yield by 22 (pot) and 16% (field), 13 (pot) and 14% (field), 12 (pot) and 6% (field) and 8 (pot) and 6% (field) over urea with DAP. While comparing the impact of all treatments on seed yield, the grain yield of pea plants for instance recorded for field trials following inoculation or fertilizer application increased in the order: *P. putida* +

Rhizobium>urea+DAP=urea+*P.putida*>DAP+*Rhizobium*>*Rhizobium*>*Pseudomonas* >urea>*DAP*. No significant impact of any treatment on grain protein of any legume was observed except some legumes where marginal increase was noticed following microbial inoculation. All the measured parameters were strongly and positively correlated.

In conclusion, the mixed inoculations of plant growth promoting rhizobacteria with exceptional qualities as observed here, especially the N_2 fixers and P solubilizers, improved the plant vitality, grain quality and showed a dramatic increase in grain yield of chickpea, pea, greengram and lentil, both under pot and filed conditions. Since legumes require a considerable amount of important but scarce plant nutrients, inoculation with favorably interacting PGPR strains are likely to provide an inexpensive alternative to chemical fertilizers for raising the overall performance of chickpea, pea, greengram and lentil, in different production systems. Moreover, this microbial approach if implemented properly will help to reduce toxicities of chemical fertilizers to soil fertility, waters and foods across different ecological niches.